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Prevalence of Tuberculosis in Cattle Slaughtered at Maiduguri Central Abattoir, Nigeria

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ABSTRACT

Bovine tuberculosis is an important public health and economic disease in Nigeria. This study reports the prevalence of tuberculosis in cattle slaughtered at the central abattoir in Maiduguri, Nigeria. Post mortem examination of 664 cattle carcasses was done at the abattoir for 6 months. The lesions collected were processed by using NALC-NaOH and subjected to acid-fast staining and microscopy. The overall prevalence of tuberculosis based on gross tubercle lesions in cattle slaughtered at Maiduguri abattoir was 62 (9.3%). Female cattle 43 (16.7%) had significantly (p = 0.0001) higher prevalence of tubercle lesions than male cattle 19 (4.7%). Cattle with thin body condition had 44 (21.2%), optimal body condition 16 (5.8%) and overweight cattle 2 (1.1%). There was significant (p = 0.0001) difference between body condition and occurrence of tubercle lesions. Prevalence of tubercle lesions in adult cattle was 29 (17.7%), young adults (25 (7.8%) and calves 8 (4.3%). Tubercle lesions were most common in thoracic lymph node and lungs than other organs. The prevalence of tuberculosis by Ziehl-Neelsen microscopy was 6.2% (41/664). There was significant (p=0.0001) differences in the prevalence of tuberculosis by Ziehl-Neelsen microscopy and demographic variable except for breeds. Tubercle lesion is endemic in cattle slaughtered in Maiduguri central abattoir. One Health approach is recommended to prevent zoonotic transmission to humans and economic losses among farmers and butchers.

Keywords: Bovine tuberculosis; Body condition; Cattle; Maiduguri; Nigeria

INTRODUCTION

Mycobacterium bovis is an acid-fast bacillus that belong to the group *Mycobacterium tuberculosis* complex (MTbC). Members of the MTbC are responsible for tuberculosis in animals and zoonotic tuberculosis in humans (Schrenzel, 2012). *M. bovis* has the widest host range of any member of the pathogenic mycobacteria (Morris *et al.*, 1994; Schrenzel, 2012). It is the cause of tuberculosis in warm-blooded animals and zoonotic tuberculosis in human (O'Reilly and Daborn, 1995; Suzuki *et al.*, 2010; Pesciaroli *et al.*, 2014;).

Taxonomically, *Mycobacterium bovis* belong to the phylum Actinobacteria, Order Actinomycetales, Family Mycobactriaceace, and Genus *Mycobacterium*. It is structurally more related to Gram-positive bacteria. However, mycobacteria do not fit into the Gram-positive category as molecules attached to the cell wall are distinctively lipids rather than proteins or polysaccharides. Frequently, mycobacteria does not retain the crystal violet and appear as "ghost" after Gram staining (Koch and Mizrahi, 2018). Like other members of the MTbC, *M. bovis* under favourable laboratory conditions divides every 12 – 24 hours. This pace is externally slow compared to that of most cultivable bacteria which duplicate at regular intervals ranging from about 15 minutes to one hour (Gormley *et al.*, 2014). The low multiplication rate of the MTbC couple with its infectious nature makes it very difficult to culture the organism under normal laboratory condition (Chauhan *et al.*, 2006; Jensen*et al.*, 2008; Malama *et al.*, 2014).

The pathology of *M. bovis* is similar to *M. tuberculosis* in human, causing chronic debilitation, coughing and further spread to other organs (Taylor *et al.*, 2007, 2003) and in a cow (Garnier *et al.*, 2003). Infected cows develop mycobacterial mastitis, causing the shedding of mycobacteria in milk leading to zoonotic transmission to human following the consumption without pasteurization (Garnier *et al.*, 2003). Most developed countries have controlled tuberculosis in animal to the barest minimum (De La Rua-Domenech, 2006; Awah Ndukum *et al.*, 2010). However, the disease is still a grave challenge in most African countries, including Nigeria (OIE, 2017).

Epidemiologically, tuberculosis is worldwide in distribution with an estimated incidence rate of 9.4 million human cases

in 2009, more than any at other time in the history of the disease (WHO, 2011); and bovine tuberculosis accounted for about 5% - 10% of human tuberculosis (Njanpop-Lafourcade *et al.*, 2001). Tuberculosis in humans due to *M. bovis* is both clinically and pathologically indistinguishable from cases caused by *M. tuberculosis* (De La Rua-Domenech, 2006). In Nigeria, reporting of bovine tuberculosis is not mandatory, and there is no active tuberculosis surveillance program; hence the actual status of bovine tuberculosis is unknown(Cadmus *et al.*, 2006). However, few works had been carried out by individuals in Nigeria. Studies dating from the 1970s and 1980s reported the proportion of tuberculosis due to *M. bovis* in cattle to be 10% in northern states and 4% in Lagos (Alhaji, 1976; Mawak *et al.*, 2006). In other studies, Cadmus *et al.* (2006) reported 5% in Ibadan, Ejeh *et al.* (2014a) reported prevalence of 1.90% - 6.50% in Makurdi Benue state and prevalence of 8.98% - 10.33% was reported among cattle slaughtered in Yola abattoir (Ejeh *et al.*, 2014b).

Transmission of tuberculosis from animals to humans mostly occur through consumption of unpasteurised milk, closed contact with infected animals (Michel *et al.*, 2010) and airborne transmission (Vekemans*et al.*, 1999). The epidemic of HIV infection in developing countries, particularly countries in which *M. bovis* infection is present in animals, and the conditions favour zoonotic transmission, could make zoonotic tuberculosis a severe public health threat to persons at risk (Darbon and Grange, 1993; Grange and Yates, 1994; Cosivi *et al.*, 1995; Moda *et al.*, 1996).

The diagnosis of bovine tuberculosis in developing countries relies on gross examination. Scanty work using bacteriological methods to determine the relative contribution of *M. bovis* and *M. tuberculosis* (Ejeh *et al.*, 2014a; Idigbe *et al.*, 1986; Malama *et al.*, 2014). This study determined the prevalence of bovine tubercle lesions in cattle slaughtered at Maiduguri central abattoir.

MATERIALS AND METHODS

Study Design

A cross-sectional study with purposive sampling of tubercle lesion in slaughtered cattle was carried out in Maiduguri Central Abattoir from June 2017 to February 2018.

Determination of Sample Size

A sample size of 664 cattle was determined at a 95% confidence interval using the method described by Thrushfield (1995) by using the formula:

$$n = \frac{Z^2 pq}{d^2}$$

Where n = sample size

q = 1 – p

p = expected prevalence of 12.27%

(Aliyu *et al.*, 2009)

d = desired absolute precision = 0.05

z = appropriate value for the standard normal deviate for the desired confidence = 1.96

$$n = \frac{1.96^2 \times 0.1227\ (1 - 0.1227)}{0.05^2} \times 100$$

$$= \frac{3.8416 \times 0.1227 \times 0.8773}{0.0025}$$

= 165.411 =165 (Minimum sample size)

However, 664 samples were examined for the prevalence of tubercle lesions in cattle. The four-fold increase in sample size was to increase the chances of positive cases.

Postmortem Examination of Carcasses

Postmortem inspection of the carcasses was carried out carefully by examination of the lymph nodes of the heads, thoracic cavities, abdominal cavities, and others. The lungs lobes, liver, spleens, hearts and kidneys were carefully inspected for lesions suggestive of BTB by palpation, visual examination and incision with a sharp knife as previously recommended (OIE, 2006). Body condition score (BCS) was based on 1-9 body condition scoring system (Soares and Dryden, 2011) with modification according to Heidi, (2017).

Sample Collection

Samples collected following postmortem examination include lymph nodes, lungs, kidneys, heart, liver, and spleen. The lesions were collected into polythene bags and labelled accordingly. Lesions collected were transported to the laboratory and were frozen until assayed. Data on the sampled animals such as age, sex, breed, predilection size, among others were documented.

Ziehl-Neelsen Microscopy

The processing of lesions was based on the OIE recommendation for digestion and decontamination procedures. The tissues were first homogenised by using a pestle and mortar (OIE, 2009), followed by decontamination in a 15ml centrifuge tube containing equal amount of homogenised specimen and NALC (N- acetyl- l – cysteine) NaOH (containing 4%NaOH, 2.9% sodium citrate). The tube containing the mixture was allowed to stand for 15 minutes at room temperature until the specimen was digested followed by neutralisation using 6ml phosphate buffer. The mixture was then centrifuged at 3000 × g for 15 min. The supernatant was carefully decanted; 2ml of phosphate buffer was added to resuspend the sediment. Finally, smears of the homogenates of each specimen were made on slides and were stained by the Ziehl-Neelsen (Z-N) method as described by Elmer (1992). The presence of acid-fast bacilli following microscopy was suggestively positive for BTB.

Data Analyses

Data analyses was done using SPSS version 17.0. for windows. Chi-square test was used to determine the possible association between BTB prevalence and sex, age, breed and body condition scores. Data obtained were presented in tables and graph. P value < 0.05 was considered significant.

RESULTS

Distribution of Gross Tubercle lesions in Cattle Slaughtered in Maiduguri Municipal Abattoir

Gross pathological investigation of lesions suggestive of bovine tuberculosis in Maiduguri Municipal abattoir revealed an overall prevalence of 9.3%. Female cattle had significantly (χ^2 = 23.673; P = 0.0001) higher gross bovine tubercle lesions than male (16.7 vs. 4.7). Cattle with poor body condition (thin) had the highest rate of bovine tubercle lesions than those with overweight or optimal body condition. The differences were statistically significant (χ^2 = 47.371; P < 0.0001). Adult cattle had the highest rate of tubercle lesions than young adult and calves. The differences were statistically significant (χ^2 = 17.394; P= 0.0001). There was no significant difference in the detection rate of tubercle lesion among the different breeds of cattle investigated at Maiduguri Municipal abattoir. However, wadara and white Fulani breeds had more tubercle lesions than gudali breed (Table 1).

Distribution of Tubercle lesion in organs of cattle inspected at Maiduguri abattoir

Sixty-two of the 664 carcasses inspected had tubercle lesions suggestive of bovine tuberculosis. Visible gross TB lesions were more in thoracic lymph nodes 31 (30.1%) followed by lungs 20 (19.4%). Visible gross tubercle lesions were less common in the diaphragm 3 (3.0%) peritoneum 4 (3.9%) and heart 5 (4.9%) had less gross tubercle lesion (Figure 1 and 2).

Table 1: Distribution of Tubercle Lesions among Cattle Carcasses Inspected at Maiduguri Municipal Abattoirs

Parameters	No inspected	Gross Lesions (%)	χ^2	P-Value
Sex				
Male	407	19 (4.7)	25.673	0.0001
Female	257	43 (16.7)		
BCS				
Thin	208	44 (21.2)	47.371	0.0001
Optimal	276	16 (5.8)		
Overweight	180	2 (1.1)		
Age				
Young	180	8 (4.4)	17.394	0.0001
Young adult	320	25 (7.8)		
Adult	164	29 (17.7)		
Breeds				
Wadara	366	38 (10.4)	1.638	0.2006
White Fulani	110	11 (10.0)		
Gudali	188	13 (6.9)		
Total	**664**	**62 (9.3)**		

Table 2: Ziehl-Neelsen Microscopy of Tissues Sample Collected from Cattle Slaughtered in Maiduguri Municipal Abattoir

Parameters	No Inspected	Ziehl-Neelsen Microscopy (%)	χ^2	P-Value
Sex				
Male	407	12 (2.9)	18.893	< 0.0001
Female	257	29 (11.3)		
BCS				
Thin	208	31 (14.9)	40.437	< 0.0001
Optimal	276	8 (2.9)		
Overweight	180	2 (1.1)		
Age				
Young	180	3 (1.7)	28.361	< 0.0001
Young adult	320	14 (4.4)		
Adult	164	24 (14.6)		
Breeds				
Wadara	366	27 (7.4)	2.042	0.3602
White Fulani	110	5 (4.6)		
Gudali	188	9 (4.8)		
Total	**664**	**41 (6.2)**		

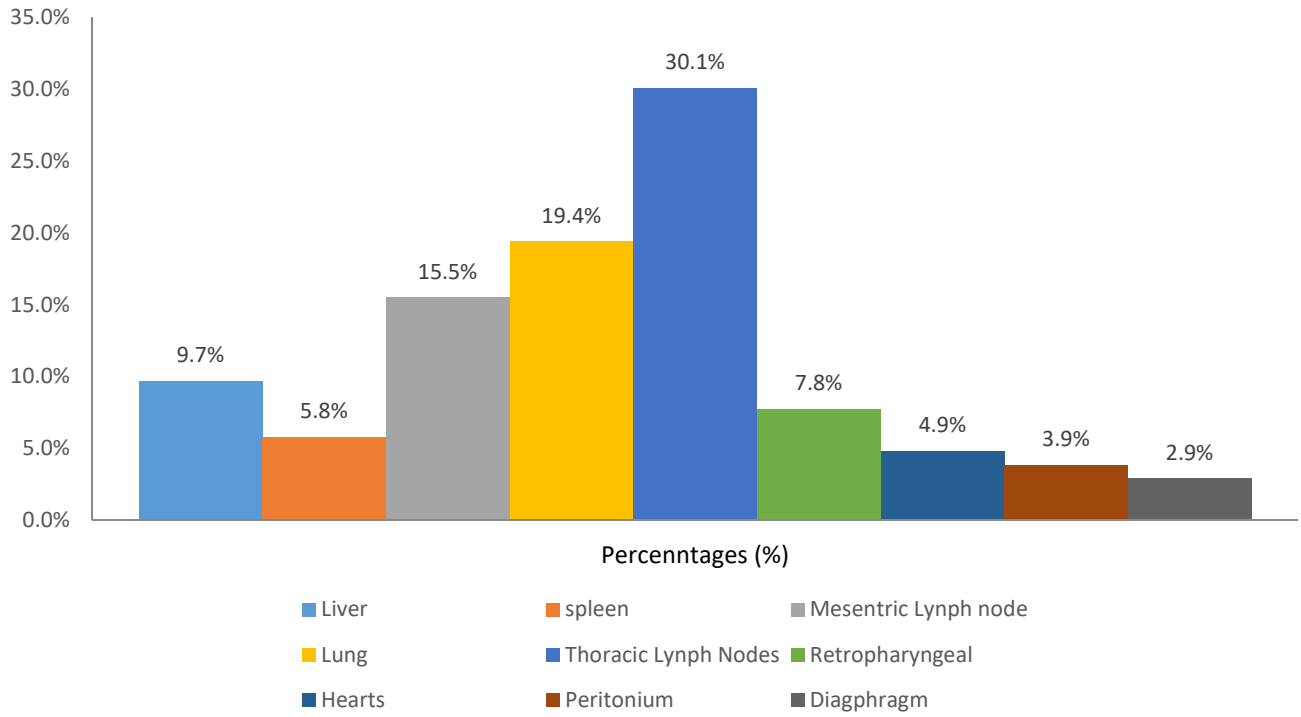

Figure 1: Distribution of Tubercle Lesion in Organs of Cattle Inspected at Maiduguri Abattoir

Detection Rate of Tubercle Bacilli by Ziehl-Neelsen Microscopy of Gross Tubercle Lesions

Table 2 showed the prevalence of tubercle bacilli by Z-N microscopy of gross tubercle lesions. The overall prevalence of tuberculosis by Z-N microscopy was 6.2% (41/664) of the total cattle examined. Female cattle had a higher prevalence of 29 (11.3%) by Z-N microscopy than male cattle 12 (2.9%). The differences were statistically significant (χ^2 = 18.893; P < 0.0001). Cattle with thin body condition, 31 (14.9%) had significantly (χ^2 = 40.437; p < 0.0001) higher prevalence of tuberculosis compared with optimal 8 (2.9%) and overweight 2 (1.1%) cattle. Adult cattle had a higher prevalence of tuberculosis than calves and young adults. The differences in the detection rate of tubercle bacilli among the age categories were significantly different (χ^2 = 28.361; P = 0.0001). There was no statistical difference in the prevalence of tuberculosis among the different breed categories of cattle examined (χ^2= 2.042; p = 0.3602).

DISCUSSION

The meat inspection system consists of an examination of the split carcasses and the organs as well as the associated lymph nodes. Abattoir meat inspection aims to ensure that animals slaughtered are wholesome and fit for human consumption. Moreover, abattoir meat inspection has provided useful epidemiological information on animal and zoonotic diseases such as bovine tuberculosis in different parts of the World (Yohanna *et al.*, 2008;Jenkins *et al.*, 2011; EFSA, 2016; Adesokan *et al.*, 2019).

In this study, the overall prevalence of gross bovine tubercle lesions in cattle slaughtered for human consumption was 9.3% (62/664). Also, the overall prevalence of tuberculosis by Z-N microscopy was 4.2% (41/664). The high prevalence of tuberculosis by identification of gross bovine tubercle lesions and Z-N microscopy in this study may be due to the absence of visible control measure against tuberculosis in animals in Nigeria; such as test and cull or test and segregation as was done in Canada, United State of America, Europe and Australia (Cosivi *et al.*, 1998; Palmer, 2007). Similarly, the absence of compensation for condemned carcasses discourage butchers and meat traders from allowing thorough meat inspection (Adesokan *et al.*, 2012; Ejeh *et al.*, 2014c). This practice has serious public health implication because carcasses containing tubercle lesions will be allowed into the food supply chain, and the public will be at risk of zoonotic tuberculosis.

The prevalence observed in this study was higher than previous studies conducted in Maiduguri Municipal abattoir. Such study includes that of Igbokwe*et al.*, (2001) reported a prevalence of 2.5% in a five years retrospective study of abattoir meat inspection, Aliyu *et al.*, (2009) reported a prevalence of 4.5% in another five years retrospective study of bovine tubercle lesions while Abubakar *et al.*, (2011) reported a prevalence of 2.7% in a ten years abattoir investigation of bovine tubercle lesions. Lower prevalence was recorded in other cities in Nigeria. Ejeh *et al.*, (2014a) reported a prevalence of 1.9% in a five years retrospective study in Makurdi, Benue State. In Yola, a prevalence of 8.68% was reported in a five years retrospective study of bovine tubercle lesions (Ejeh *et al.*, 2014b). Conversely, the prevalence observed in this study is lower than the report of Aliyu *et al.*, (2009) in Gombe abattoir in which prevalence of 12.5% was observed in a five-year retrospective study of abattoir meat investigation of bovine tubercle lesions.

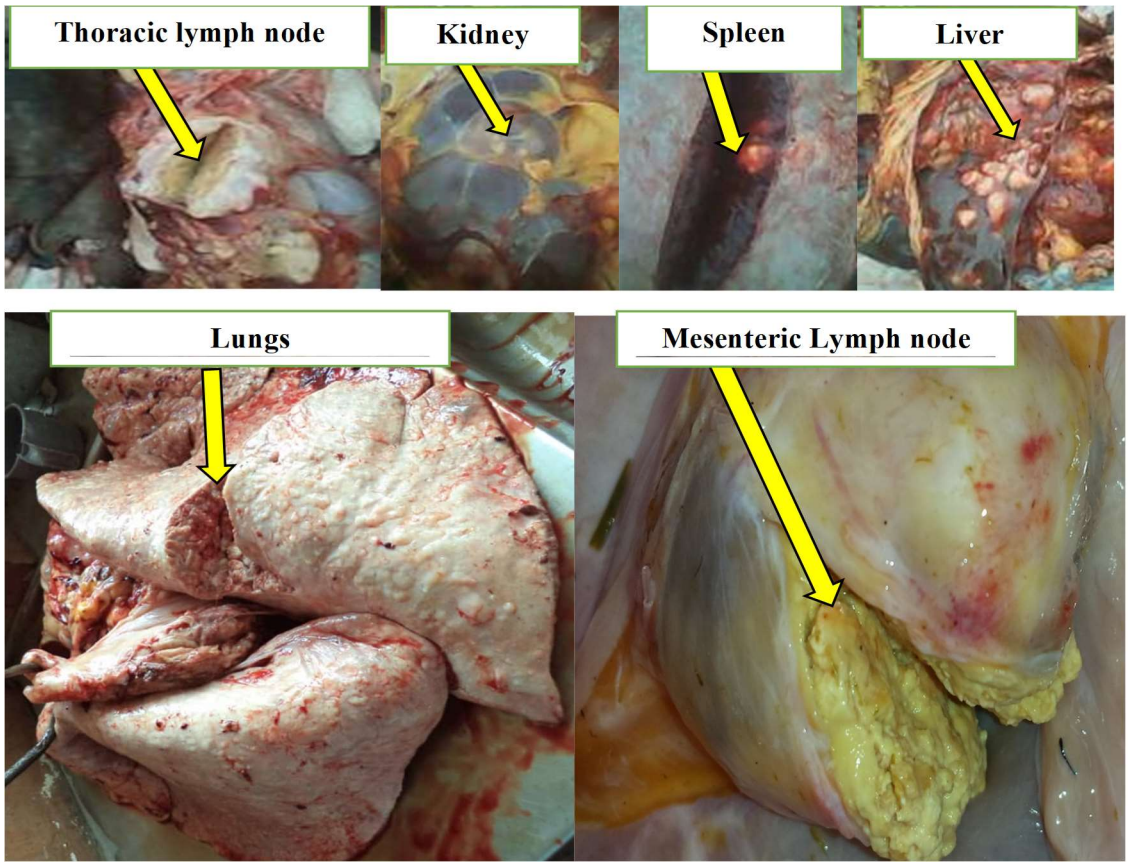

Figure 2: Photographs of Tubercles in Visceral Organs

The difference between the prevalence of bovine tubercle lesions in this study and previous study in Maiduguri Municipal abattoir may be due to an increase in the incidence of tuberculosis in cattle resulting from lack of adequate control measures (Cadmus *et al.*, 2006; Adeshokan *et al.*, 2019). Also, thorough abattoir meat inspection applied during this research may explain the reason for the high prevalence of bovine tubercle lesions observed compared to previous studies which were based on abattoir records of meat inspections. Retrospective data are associated with problems such as missing data, inaccuracies and under reporting (Aliyu *et al.,* 2009; Ejeh *et al.,* 2014b).

The prevalence of tuberculosis in this study was higher among cows than bulls. It was also observed that adult cattle had the highest prevalence of tuberculosis than younger ones. This could be attributed to the fact that female cows are kept for longer period by farmers for breeding purpose than male cattle, and since tuberculosis is a chronic disease, its prevalence had been reported to be higher among adult cattle than the younger ones. The finding was similar to previous reports that tuberculosis was more common among female and adult cattle (Opara *et al.*, 2012; Ejeh *et al.*, 2014c; Kwaghe *et al.*, 2015; Jajere *et al.*, 2018).

The finding of this study showed that the prevalence of tuberculosis was higher among cattle with poor body condition than optimal and overweight cattle. It further substantiates the fact that tuberculosis is a chronic debilitating disease resulting in progressive emaciation of infected cattle (Corner *et al.*, 2011; Fentahun and Luke, 2012).

The difference between the prevalence of tuberculosis by gross tubercle lesions and Z-N microscopy may be because some tubercle lesions observed in this study may be as a result of infections by other pathogens such as *Norcadia, Corynebacterium, Rhodococcus, Streptococcus* and fungi (Neill *et al.*, 2001; Rastogi *et al.*, 2001). The findings of this study was an improvement in the report of bovine tuberculosis in the study location. Previous studies (Igbokwe *et al.,* 2001; Aliyu *et al.*, 2009) were based on retrospective abattoir records of meat inspection which may be associated with inherent problems such as poor record keeping.

Conclusion

The prevalence of bovine tuberculosis in this study were 9.3 % and 6.2 % by gross tubercle lesion and acid-fast microscopy, respectively. Tubercle lesions were found to be more frequent in the lymph nodes of the thoracic cavity than other regions in the body, suggesting respiratory route transmission. Public health measures such as continuous education of the abattoir workers and animal handler on the control and prevention of zoonotic tuberculosis that may originate from animals in this abattoir.

Competing Interest

The authors declare that they do not have any conflict of interest.

Author's Contribution

FAL conceived and designed the study. FAL; FEE; AW; CNK; KBK; HMK conducted research, provided research materials, and collected and organized data. FAL; FEE analyzed and interpreted data. FAL; FEE; AW; CNK; KBK; HMK wrote the initial and final draft of the article, and provided logistic support. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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