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Research Article

Estimates of Mycobacterial Infections Based on Abattoir Surveillance in Two North-Eastern States of Nigeria

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Abstract

Tuberculosis (TB) is recognized as one of the most important threats to animals and human health causing morbidity, mortality and economic loss. It remains a major global health problem in livestock and man. In livestock, *M. bovis* is the most pathogenic species among the members of the *Mycobacterium tuberculosis* complex. It is widely distributed worldwide causing significant economic losses. In this study, we aimed at determining the prevalence of Mycobacteria in bovine carcasses in two north eastern states of Nigeria. The study was a cross-sectional study carried out at six selected abattoirs in the two states. Animals with lesions that were compatible with tuberculosis were recorded. Observations of pathological lesions of such organs as mediasternal, retrophargyngeal, submandibular, tracheobronchial lymph nodes, and, lungs, liver and intestines were also made. In Bauchi, 8,497 (9.8%) were found to be positive for gross TB lesions, while 10,505 (13.7%) were found to have TB lesions in Gombe State. Large proportion (50%) of TB lesion in both states was recorded in the respiratory pathway followed by digestive system (21%) and pre-scapular lymph nodes (18.6%). Tissue samples (300 from both states) were collected and cultured on Lowenstein Jensen media (containing either glycerol or pyruvate), 155 (51.7%) were Acid fast bacilli (AFB) positive and 82 (52.9%) were positive on culture and 15 (18.3%) were positive on Bioline® analysis. All isolates which were positive on Bioline® analyses were subjected to Genotype® MBTC analysis, (Hain assay PCR analysis) in order to differentiate them into their various species. The MBTC analysis showed, 12 (80.0%) to be *Mycobacterium bovis*, 2 (13.3%) *Mycobacterium tuberculosis* and 1 (6.6%) as unidentified using the standard banding patterns. There is also, the need to strengthen routine meat inspection and also to step up public health awareness programs on zoonotic nature of TB particularly among the abattoir workers and the herdsmen.

Keywords: Bovine Tuberculosis; *Mycobacterium bovis; Mycobacterium tuberculosis;* Abattoir; Hain Assay; Culture

Introduction

Bovine tuberculosis (bTB) is primarily a disease of cattle caused by Mycobacterium bovis characterized by formation of tubercles in tissues/organs of the affected animal [1-4]. The disease has been reported in 176 countries of the world as one of the important bovine diseases causing great economic loss [5]. It occurs primarily in cattle but also causes tuberculosis in most mammals including humans [6]. In cattle, infection can result in a chronic disease that affects animal welfare and productivity in some countries leads to significant economic losses by causing morbidity and mortality [7]. In the developing countries, unfortunately, bTB has received little attention by way of surveillance, control and or eradication programme. Thoen [8] reported that in the developing countries nearly 85% of cattle and 82% of the human population live where the disease is prevalent and or only partially controlled. Nevertheless, the available information is limited due to inadequate disease surveillance and lack of better diagnostic facilities [6,9]. In particular, information on genotypic characteristics of M. bovis, a strain affecting the cattle population in Nigeria, is limited [10]. Such information is critical to monitor transmission and spread of the disease among cattle [11]. In Nigeria, bTB is considered an endemic disease, and has been reported in many parts of the country [12-15].

In Nigeria, bovine tuberculosis is diagnosed mainly by post mortem meat inspection of carcasses in government-controlled abattoirs. The method detects lesions that are at advanced stages of the infection. Pathological changes are observed when the immune response attempts to sealed off the infection leading to the development of lesions that are visible in the lymph nodes that drain the head and respiratory system [16]. However, it has been observed that there have not been any serious attempts in sampling the major cattle producing regions of Nigeria or to type all the species cultured in order to differentiate the mycobacterial species circulating in the country and also the factors associated with the disease in the country. The main objectives of this study were to elucidate mycobacterial species prevalence and identify factors associated with the disease in cattle.

Materials and Methods

Study population

The study was conducted in Bauchi and Gombe States, North-Eastern, Nigeria. Bauchi State lies between latitudes 10°10' and 10°33N and longitudes 9°40' and 10°13'. The climate of the states is Semi-arid characterized by a long dry season. The climatic variables vary considerably during the year and are eratic. The total annual rainfall ranges from 600mm in the north to 1000mm in the

Southern part of the state. Most of the state falls within the Sudan Savannah vegetation belt, but traces of Guinea Savannah vegetation are found in the parts of the southern districts.

Gombe state is located between latitude 9°30′ and 12°30′N and longitudes 8°45′ and 11°45′E of the Greenwich Meridian (Anon, 2007). The state is in the North-eastern part of Nigeria, with its capital at Gombe town. The cattle population has been estimated at 800,000 heads of cattle according to vaccination records of the Ministry of Animal Husbandary and Nomadic Affairs, Gombe State.

Study Design

This study was a cross-sectional study which consisted of two parts: a field study in Gombe and Bauchi States followed by laboratory and molecular analyses carried out in Zankli Tb research laboratory Abuja, Nigeria. The field study was composed of primarily meat inspection in selected abattoirs in each of state. Animals were cast for slaughter data pertaining the owner/butcher; sex, age, and body condition were recorded. Meat inspection was carried out following standard meat inspection procedures. Tissue samples were stored in Cetyl Pyrinidium Chloride (CPC) solution and transported to the Zankli Hospital Tuberculosis Reference Laboratory (TBRL) in Abuja.

Mycobacterial culture

Tissue samples were obtained at the selected abattoirs. All the cattle were necropsied by routine technique as described by Monaghan., *et al.* (1994) [34]. In cattle with macroscopic lesions, the lesioned material was collected. If no lesions detected, samples were taken from mediasternal, retropharyngeal and bronchial lymhnodes and from the lung. These samples were numbered and then kept in sterile containers and frozen at -20°C until processed. Tissues were divided into equal portions. One half of each of the sample was kept frozen for confirmatory analysis in case the culture needs to be repeated. All samples were thawed at room temperature and processed as follows. Fat and fibrous tissues were removed by aseptic technique and the surface of each sample was examined for gross lesions. Approximately 5g of the remaining tissue was cut into small pieces with a sterile scapel blade. These pieces of tissues were homogenized using a blender in the Biosafety cabinet with 3 ml of sterile distilled water. Two millilitres of the homogenate were separated for decontamination by the addition of 3 ml of a solution of 1% sodium hydroxide/3% lauryl sulphate (w/v), after which they were incubated for 30 minutes. The mixture was then neutralized with 8.75% (w/v) orthophosphoric acid by using bromocresol blue as an indicator.The neutralized suspension was then centrifuged at 3500 rpm for 30 minutes. The supernatant was discarded, and 0.25 ml of the pellet was inoculated unto Loewenstein-Jensen media with and without pyruvate. The media were checked for growth weekly, for 5 to 8 weeks. Tubes with no evidence of growth were recorded as negative and discarded. The original samples were recultured if the culture media became contaminated during the incubation time. Colonies suspected of being *Mycobacteria* were examined for the presence of acid-fast bacilli by Ziehl-Neelsen stain technique. All acid-fast bacilli positive samples were stored for further identification and molecular analysis.

SD TB AgMPT64 (SD BiolineR)

From the suspected colonies, three colonies were removed and emulsified in distilled water. 100 µl was then added to the well, the cassette was kept undisturbed at room temperature inside the biosafety cabinet. The inoculated cassette was then examined at the end of 15 minutes post inoculation for the presence of a pink band in the control and the test region. The validity of test was confirmed by the presence of a band. If the band is only in the control but absent in the test the result is considered as negative, confirming the presence of Non-Tuberculous *Mycobacteria*, while the presence of a band in both control and test indicates positive result.

Typing of acid fast bacilli (AFB)

Hain GenoType MTBC (Mycobacterium tuberculosis complex) assay kits (Hain Lifescience, GmbH, Nehren, Germany) was used to type all cultures that were found to be positive. Another kit known as the GenoLyse kit (Hain Lifescience, GmbH, Nehren, Germany) also was used to extract the DNA from cultures that were positive based on the instructions by the manufacturers. The Hain GenoType MTBC assay was performed following the manufacturer's instructions and as previously described [18]. 45 µl of a PCR mix containing 10 µl of amplification mix 'A' (containing biotinylated primers) and 35 µl of amplification mix 'B' were added into 0.2 ml PCR tube. The extracted DNA (5 µl) was added to make a final reaction volume of 50 µl. Amplification was carried out in a thermal cycler(Applied Biosystem, 2720 Thermal cycler, USA) using the following protocol: denaturation at 95°C for 15 minutes; 10 cycles of denaturation at 95°C for 30 seconds and elongation at 58°C for 120 seconds; an additional 20 cycles of denaturation at 95°C for 25 seconds, annealing at 53°C for 40 seconds, and elongation at 70°C for 40 seconds; and a final extension at 70°C for 8 minutes. Twenty microliters of the resulting amplification products were hybridized on labelled membrane strips at 45°C for 30 minutes. Hybridized products were developed by addition of a conjugate buffer (containing streptavidin conjugated with alkaline phosphatase) and a subsequent addition of the substrate buffer for colorimetric detection of the bands. The various species were then identified using the interpretation chart provided by the manufacturers(http://www.hain-lifescience.de/en/product/mycobacteria/genotype-mbtc.html).

Data Presentation and Analyses

Data were presented in the form of Tables, Figures, and Plates. For Hain tests, evaluation sheets with pasted strips in the designated fields were read by aligning the conjugate bands and universal bands with their respective lines on the sheets and positive signals on columns were then used to determine the species with the help of the interpretation chart (Appendix I).

Ethical statement. This study involves the use of cattle that were slaughtered. Local approval was obtained from the Director Veterinary services at the Ministry of Agriculture/Animal Husbandry.

Results

Sampling of Bovine Tissues from Abattoir

A total of 300 tissue samples from Bauchi and Gombe States were collected from carcasses during postmortem examinations in the designated abattoirs (Table 1). Forty five percent (45%) of the samples from Bauchi were from male carcasses while the remaining 55% were from females carcasses, similarly 35% of the carcasses from Gombe State were from male carcasses with the remaining 65% from females. Furthermore 35 (17.5%) of the samples collected were from animals less than 4 years old, while, the remaining samples 165 (82.5%) were from animals older than 4 years old (Table 1). There was no significant association between the positive samples obtained and the age group as well as that sex of the animals and positive samples for TB (P > 0.05) (Table 1).

Variable	Total Number collected (for both states)	Number positive (%) (for both states)	Chi-square	p-value	OR (95% CI)
Age (years)					
≤ 4	35	10 (28.6)	7.792	0.005	1 (REF)
> 4	165	25 (15.15)			3.020 (1.357 - 6.723)
Total	200	35 (17.5)			
Sex					
Male	80	18 (9.0)	0.849	0.357	1 (REF)
Female	120	34 (17.0)			0.734 (0.380 - 1.418)
Total	200	52 (26.0)			

Table 1: The Level of Association of Sex and Age of Animals with Tuberculous Lesion in Bauchi and Gombe States, Nigeria.

Prevalence of Organ Distribution of Bovine Tuberculosis in Bauchi and Gombe States

Based on the postmortem examination conducted on 86,702 cattle in Bauchi and 76,639 cattle in Gombe abattoirs, the prevalence of bTB suspected lesions was 9.8% in Bauchi and 13.9% in Gombe (Table 2). The lesions were of different sizes in various organs. The organ level distribution of lesions is indicated in table 3. Highest proportion of TB lesions was recorded in the respiratory pathway with lungs having 35% in Bauchi and 30.6% in Gombe, followed by the mesenteric lymph node (LN) with 25.0%, submandibular lymph had 23% in Bauchi and 12.5% in Gombe, while the least were seen in others (Heart, intestines and liver) with 6.5% and 8.20% for Bauchi and Gombe respectively. There were no suspected TB lesions found in the muscles in both states. The monthly incidence of suspected Tb lesions at the abattoirs under study is presented in figure 1, indicating the number of various lesions collected and the sequence of occurrence for number of animals slaughtered and lesions observed during the year observed against time is presented in figure 2. Most lesions in the lungs were observed at the parenchyma (Plate 1). From the study; it was observed that the seasonal index for lesions in the abattoirs in Gombe State had peaks in February and July with lesser peaks in April and November (Figure 1).

State	Location	Cattle slaughtered	No. with Suspected TB lesions	Percentage (%)
Bauchi	Bauchi	65,381	4,932	7.5
	Misau	9,546	1,265	13.3
	Azare	11,775	2,296	19.5
	Total	86,702	8,497	9.8
Gombe	Gombe	57,943	7,010	12.09
	Bajoga	1,750	300	17.14
	Billiri	16,946	3,195	18.85
	Total	76,639	10,505	13.9

Table 2: Summary of Slaughter House Survey for Suspected TB Lesion in the study area.

Organs inspected	No. of tb lesion	Percentage (%)
Bauchi (n = 5671)		
Lung	1985	35.0
Mesanteric LN	1452	25.6
Submandibular LN	1304	23.0
Mediasternal LN	414	7.3
Others (heart, intestine, liver etc.)	369	6.5
Tracheobronchial LN	147	2.6
Gombe (n = 8112)		
Lung	2485	30.63
Mesentaric LN	1703	21.0
Mediasternal lymph node	1334	16.44
Submandibular LN	983	12.12
Tracheobronchial LN	942	11.61
Others (heart intestine, liver etc.)	665	8.2

Table 3: Organ Level Distribution of Suspected Bovine Tuberculosis Lesions found during Abattoir Inspection.

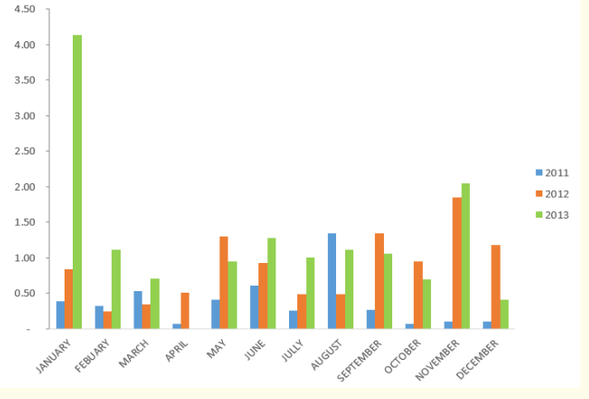

Figure 1: Monthly Indices of lesions observed at the Gombe Abattoir between the period of 2011-2014.

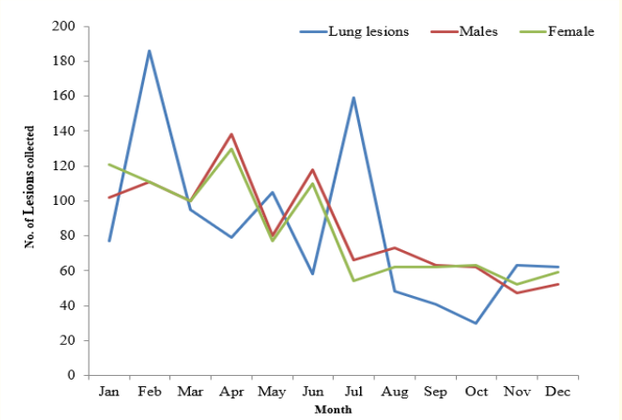

Figure 2: Sequence of occurrence of number of animals slaughtered and lesion observed during the years (2011-2014).

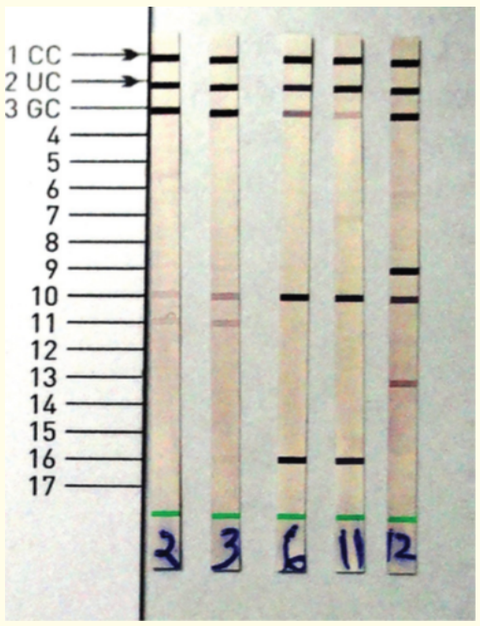

Plate 1: Results showing banding patterns in Hain Assay Test.

Culture and isolation of *Mycobacteria* from Bovine Tissues from the abattoirs

Out of the 300 samples collected and cultured, 210 (70.0%) were negative or had no growth on them after 8 weeks of incubation, while 78 (26.0%) were positive based on cultural characteristics and Zeihl-Neelsen Stain technique. Approximately, 12 (4.0%) of the culture positive samples were found to be contaminated (Table 4).

This analysis yielded 28 as MTBC, while the remaining seven were characterized as NTM (Table 4). Of the 78 positive samples by AFB, 28 (35.9%) were found to be positive for MTBC following subjection to Bioline analysis, the remaining were characterized as NTM (Table 4).

Type of specimen	No. of samples collected	AFB Positive (%)	Culture Positive (%)	Bioline Positive (%)
Tissue	300	78 (26.0)	25 (8.3)	28 (9.3)

Table 4: Specimen Reactivity to Various Tests (Smear, Culture and Bioline ®) Positivity.

Isolation and identification of Mycobacterial isolates using HAIN Assay

Of the 28 *Mycobacterium tuberculosis* complex (MTBC) isolates from the tissues, 15 (5.4%) were found to be *Mycobacterium bovis (M. bovis),* 8(2.9%) were *Mycobacterium tuberculosis* (M.tb) and the remaining 5 (1.9%) were unidentified using the standard banding patterns (Plate 2).

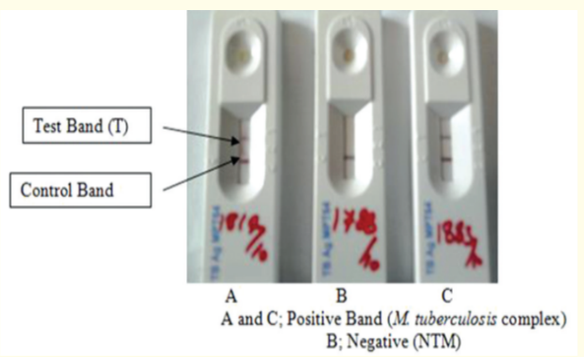

Plate 1: Differentiation of *M. tuberculosis* complex and non-tuberculous *Mycobacteria* by BIO-LINE SD Ag MPT64 tuberculosis test.

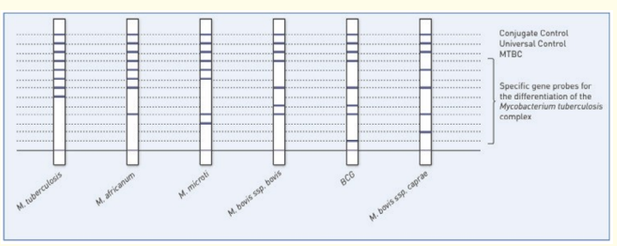

Figure 3: GenoType MTBC for differentiating the complex (adapted from the Hain Life Sciences Manual, 2012).

Discussion

Bovine tuberculosis is an important disease problem due to its effects on livestock production and its zoonotic potential. The current study has showed that there were high prevalences of bTB in cattle population and this will be having significant effects on production and animal health. Subsequently, this can lead to zoonotic transmission in cattle rearing communities, there by encouraging the disease transmission to cattle handlers' consumers of milk, and other livestock handlers [6].

From this study it was found that the prevalence of suspected TB lesions in cattle in Bauchi State was 9.80% and 13.9% in Gombe State. This is in agreement with previous reports by Aliyu., *et al.* [19] who reported 12.27% in Gombe, while Saidu., *et al.* [20] reported an abattoir prevalence level of 8.8% in Bauchi State. Similar prevalence of 8.30% was reported by Cadmus., *et al.* [21] in food animals in Nigeria. The findings of this study were higher than earlier reports of bovine tuberculosis in other parts of Nigeria. For example in Adamawa State, Aliyu., *et al.* [22] reported a prevalence of 0.34% while in Ogbomosho in Osun State, Ameen., *et al.* [22] reported a prevalence of 0.55% and Nwata., *et al.* [23] in Enugu State reported a prevalence of 1.4%. Researchers in other parts of Africa reported similar findings. For example in Cameroun, 6% prevalence was reported by Thoen., *et al.* 2009 [24], and in Chad, Milan., *et al.* [25] reported a prevalence of 9%. The reasons for the high prevalence of tuberculosis lesions in slaughtered cattle in Gombe and Bauchi abattoirs were not clear but may be due to the fact that, there is no active bovine tuberculosis control programme in Nigeria and that movement of cattle across both local and international boundaries were not restricted. This situation may promote the entrance of infected animals from neighbouring countries such as Chad, Niger Republic and Cameroun as suggested by Aliyu., *et al* [19]. Another explanation for the high prevalence of tuberculosis lesions in cattle slaughtered in Gombe and Bauchi States could be due to the absence of ante mortem examinations. During the late dry season and early rainy season, prevalence of tuberculous lesions were 12.89% in Gombe and 10.95% in Bauchi while the prevalence was lower during late rainy season (7.6%) in Gombe and 6.9% in Bauchi and early dry season respectively. These findings were different from that of Awah-NduKum., *et al.* [5] in Cameroun who reported that detection of TB like lesions was not influenced by season but high when the cattle were stressed such as during inter season and peak periods. Similarly, Ameen., *et al.* [22] reported similar findings in Ogbomosho, Nigeria. However, Bikom and Oboegbulum [26] reported strong association between season and tuberculous lesions but the reason for the difference in the seasonal variations observed in their study was not stated.

The result of the findings of this study showed there was no association between sex and tuberculous lesions. These findings agree with that of Nwanta., *et al.* [23] and Awah-Ndukum., *et al.* [5] who reported also that there was no association between sex and tuberculous lesions detected in the abattoirs.

From the study the organs found to have suspected TB lesions were similar to those reported by other workers. Finding of the highest lesions in the lungs could be that the animals acquired their infections through aerosol during grazing or at night. It could also be through addition of new animals to the herds as Kaltungo., *et al.* [27] reported pastoralists' commonly adding new animals into their herds without any quarantine. The findings of this study is in agreement with the results found by Ameni and Wudie [28] who showed that 72% of the gross lesions were in the thoracic cavity, while Regassa., *et al.* [29] and Tigre., *et al.* [30] also detected 50% and 48.4% of Tb lesions respectively in respiratory pathway.

M. bovis and *M. tuberculosis* were identified in slaughter cattle in the study area, indicating an important finding with economic and public health consequences. The finding of *M. tuberculosis* in cattle further highlights the extremely complex nature of the problem there by suggesting an anthropozoonosis (transmission between humans and cattle) in the study area. This study has also shown that cattle come down with human based TB since *M. tuberculosis* was recovered from them. Thus, the habit of pastoralists living closely with their cattle at night could account for this. Some studies conducted in the past have shown that the prevalence of *M. tuberculosis* in cattle to have always not exceeded 1% [31] but findings from this study, found the prevalence to be higher than 1%. Recent studies have however revealed that the prevalence of *M. tuberculosis* in African and Asian cattle ranges between 4.7% and 30.8% in countries with high human TB incidences [32]. The identification of *M. bovis* and *M. tuberculosis* in slaughtered cattle confirms that there is anthropozoonotic transmission in the studied areas, and this should be considered a public problem especially in the pastoral communities that have emotional attachments to their cattle for cultural, and socio economic reasons [33]. Since other controls measures were not put in place, emphasis should always be on performing constant and sustained thorough of meat inspection.

Conclusions

This study has shown that, *M. bovis, M. tuberculosis* and other NTM organisms circulating in cattle destined for human consumption in the study area. The identification of *M. bovis* and *M. tuberculosis* in cattle has shown that there is a risk of (reverse) zoonotic transmission and the need for urgent action.

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Volume 1 Issue 5 May 2018