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Molecular Epidemiology of Tuberculosis in Cattle and Human Patients in Bauchi and Gombe States, Northeastern Nigeria

[1*]Ibrahim, S., [1]Danbirni, S., [2]Saidu A. S., [1]Usman, B. A., [3]Kaltungo, B. Y. and [3]Mohammed, F. U.

[1]*Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria*
[2]*Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Maiduguri*
[3]*Veterinary Teaching Hospital, Ahmadu Bello University, Zaria*

[*] *Author for Correspondence: sihadejia@gmail.com*

ABSTRACT

Tuberculosis (TB) is recognized as the most important threat to human and animal health causing morbidity, mortality, and economic losses. A study was conducted to assess the epidemiology and zoonotic implications of TB in two states of northeastern Nigeria, using abattoir surveillance, culture, and molecular typing methods. The human sputum of in-patients from selected hospitals within the study areas was also collected, cultured, and subjected to molecular typing. Out of the 250 human sputa collected, 74 (29.6%) were positive for culture and 40(16%) were positive on Bioline® analysis. Also, of the 200 tissues collected, 62(32.5%) were AFB positive and 25(12.5%) were positive on culture and 18(9.0%) were positive on Bioline® analysis. All positive isolates on Bioline® analyses were further subjected to Genotype® MBTC analysis. A total of (n = 58) isolates; sputum (n=40) and tissue (n=18), identified as MBTC were further subjected to Genotype MBTC® Hain assay PCR analysis. in order to differentiate them into their various species. Of the MBTC isolates from the tissues, 8(4.0%) were found to be *Mycobacterium bovis,* 5(2.5%) were *Mycobacterium tuberculosis* and the remaining 5(2.5%) were unidentified using the standard banding patterns. The 40 MBTC from sputum showed 30 (75%) as *Mycobacterium tuberculosis* 6(15%) were *Mycobacterium bovis,* 3(7.5%) were *Mycobacterium africanum* and 1(0.4%) was unidentified using the standard banding patterns. In conclusion, the study revealed the public health importance of bTB in the study area. There is also the need for public health awareness programs on the zoonotic nature of bTB among the abattoir workers and the herdsmen.

Keywords: Bauchi; Cattle; Gombe; Humans; Molecular; Tuberculosis

INTRODUCTION

The members of the genus *Mycobacterium tuberculosis* complex include *Mycobacterium tuberculosis; Mycobacteriu m bovis, Mycobacterium canetti, Mycobacterium microti, Mycobacterium pennipedii, and Mycobacterium africanum.* The *M. bovis* and *M. tuberculosis* are of public health importance. *Mycobacterium bovis* is the most pathogenic species among the members of the MTBC-complex (Cosivi *et al.,* 1998). It is widely distributed throughout the world affecting all age groups of humans and animals, causing significant economic losses and posing challenges in disease control (Cosivi *et al.,* 1998). Human infection due to *M. bovis* is thought to be mainly through drinking contaminated or unpasteurized raw milk and eating the undercooked meat. The high prevalence of TB in cattle, close contact of cattle and humans, the habit of raw milk and meat consumption, and the increasing prevalence of HIV may all increase the potential for transmission of *M. bovis* and other *Mycobacteria* between cattle and humans (Shitaye *et al.,* 2007).

Although, recent studies indicated that *M. tuberculosis* has been isolated from cattle (Ameni *et al.,* 2011) and *M. bovis* from humans infected with bovine tuberculosis (Zeweld, 2014). *Mycobacterium tuberculosis* is specifically adapted to humans while *M. bovis* is most frequently isolated from domesticated cattle (Smith *et al.,* 2006). In spite of variation in host specificity, the members of MTBC are characterized by 99.9 % or greater similarity at the nucleotide level and are virtually identical at the 16s rRNA sequence (Brosch *et al.,* 2002). Therefore, this makes the clinical diagnoses in humans very difficult, due to indistinguishable clinical manifestations between the two species. Also, the emergence of multi-drug and extensively drug resistant *Mycobacterium* species poses a serious threat to public health and coupled with the huge economic losses due to prolonged treatment, prevention and control of the disease (Ejeh *et al.,* 2013; Saidu *et al*., 2015).

In sub-Saharan Africa, humans, and animals share the same microenvironment, watering points, feeding facilities, and grazing land. There is a close cohabitation between the human and cattle populations. Nevertheless, the available information is limited due to inadequate abattoir surveillance and lack of better diagnostic facilities (Cosivi*et al.,* 1998; Assegede*t al.,* 2000). In particular, information on the genotypic characteristics of *M. bovis*as well as *M. tuberculosis,* strains affecting the cattle population in Nigeria is limited (Cadmus *et al.,* 2010). Such information is critical to monitor transmission and spread of the disease among cattle (Berg *et al.,* 2011). Thus, the true prevalence of the disease in the country has been under reported. In developing countries like Nigeria, the socio-economic situation and low standard of living areas for both animals and humans are contributing more in TB transmission between human to human and human to cattle or *vice versa* (Ameni *et al.,* 2003; Ejeh *et al.,* 2013).

In Nigeria, bTB is considered an endemic disease, and has been reported in many parts of the country (Eid, 1976; Ayanwale, 1984; Du-sai and Abdullahi, 1994; Ankugah, 2002; Cadmus, 2004). Other studies in humans have shown the involvement of *M. bovis* in causing tuberculosis in man. For instance, in a study of 102 *M. tuberculosis* complex from patients with pulmonary TB, four (3.9%) were identified as *M. bovis* (Idigbe, 1986). Recently, Mycobacterium Tuberculosis Complex (MTC) strains isolated from human samples in Nigeria were characterized using molecular methods like spoligotyping, Variable Number Tendem Repeats (VNTR), Multi-Locus Sequence (MTLS), etc. Cadmus *et al.* (2006) had also reported three cases of human tuberculosis due to *M. bovis* in a study of 55 human sputum samples in Ibadan, Nigeria. Therefore, this study was designed to establish the prevalence and zoonotic linkage of bovine tuberculosis due to *Mycobacterium bovis* and reversed zoonotic transmission of human-TB due to *M. tuberculosis*, in the two selected northeastern states.

MATERIALS AND METHODS

Ethical Statement

An approval and consent of the abattoir staff were sought for and approved. The approval was obtained verbally, in form of verbal consent at individual level from the abattoir managers.

Study Area

The study was conducted in Bauchi and Gombe states of Nigeria. Bauchi State lies between latitudes 10° 10́ and 10° 33′ N and longitudes 9° 40́ and 10° 13́ E, while Gombe state is located between latitude 9° 30′ and 12° 30′ N and longitudes 8° 45′ and 11° 45′ E of the Greenwich Meridian (Census, 2006). The Bauchi State has two distinct climates, the dry season (December to March) and the rainy season (April to November) with an average rainfall of 850mm, with the mean maximum monthly temperature of 37°C, the relative humidity of 94 % in August and 10 % in December. Whereas, Gombe State lies between latitude 10.08N and 11.24E and longitude 11.02N and 11.18E. Gombe is the capital city of Gombe state which is located in the centre of the northeastern part of the country. It borders Borno and Yobe to the east and north respectively, Adamawa and Taraba to the south, and Bauchi States to the west, with a total land mass area of 20, 265 km2. The wet season is from April to October and the dry season is from November to March. Gombe has a population density of 2,353,879 (Census, 2006). Guinea Savannah grassland is the natural vegetation with some woodland. This makes Gombe a conducive grazing land and pasture for cattle rearing and predominantly an agrarian state with more than 80% of the population engaged in agricultural production.

Study Design

This study was a cross-sectional observational study which employed the abattoir tissue samples collection, human patient's sputum collection followed by laboratory and molecular analyses carried out at Ahmadu Bello University, Zaria and Zankli Tb-research laboratory, Abuja, Nigeria. The study period was from April, 2016-December, 2018.

Study Animals

Cattle slaughtered at Bauchi and Gombe major abattoirs were the subjects of the study. Their respective breeds, sexes, and ages were recorded during sampling. A convenient sampling technique was employed at the abattoirs. A minimum of 5.0-10g tissue was incised from the affected from 2-3 different sides, stored in bottles and transported to the laboratory on ice pack for further processing.

Sample Size Determination

A prevalence rate of 8.3% in a study by Saidu *et al.,* 2015 in Bauchi was used to estimate the sample size taken in this study.

A Sample size was calculated using the formula by Thrusfield (1997) at a confidence level of 95%. $n= Z^2pq/d^2$

For tissue Samples:

Where n=Sample size, Q=1-p, P=Prevalence (8.3%) (Saidu *et al.,* 2015) and d=Level of Significance (Precision) (0.05)

$$\text{Therefore, } N= \frac{1.96^2 \times (0.083 \times 0.917)}{(0.05)^2}$$

= 3.842 x 0.07611/0.0025 = 0.2924/0.0025 = 117 samples

For Sputum Samples:

Where n=Sample size, Q=1-p, P=Prevalence (4%) (Ndahi *et al.,* 2014) and d=Level of Significance (Precision) (0.05)

$$\text{Therefore, } N= \frac{1.96^2 \times (0.04 \times 0.96)}{(0.05)^2}$$

= 3.842 x 0.0384/0.0025 = 0.1475/0.0025 = 59 samples

However, for this work, a total of 200 human samples and 250 tissues in cattle were taken for more precision and accuracy.

Abattoir Inclusion Criteria

Abattoir selection was by convenience sampling based on the herd's location, the daily slaughter capacity of the abattoirs, accessibility of the abattoir to the general public and its socio-economic impact on the state. Therefore, three (3) major abattoirs from each senatorial district were selected.

Thus, Bauchi, Misau and Azare abattoirs were selected from Bauchi State, whereas, Gombe, Bajoga, and Billiri abattoirs were selected from Gombe State representing the Central, South and the Northern senatorial zones respectively.

Tuberculin Testing

Alongside abattoir sampling, cattle herds around the study area were also screened and sampled for tuberculosis using a tuberculin test screening. A Caudal-Fold Test (CFT) was employed for the screening. The first test to detect possible carriers of bovine tuberculosis is the CFT, in which a small portion of purified protein derivative (PPD) tuberculin is injected into the fold of skin at the base of the tail. The CFT is read 72+/- 6 hours later. If turned out negative, no further action is required (Norby *et al*., 2004).

Post-Mortem Examination: Abattoir Meat Inspection

Three (3) abattoirs from each state within the study areas were selected, wherein each animal meticulous post mortem examination was conducted. The calculated sample size was 59 However, a total of n=200 tissue samples suspected of tubercle lesions were collected; hundred (n=100) each from Bauchi and Gombe state. Animals with lesions that were compatible with typical tubercle lesions, with creamy-caseous and gritty sound on incision (inclusion criteria) were recorded and sampled. Observations of pathological lesions of such organs as mediastinal, retropharyngeal, submandibular, bronchial lymph nodes, lungs, liver, and intestines were recorded as described by Corner *et al*. (1990). Fresh tubercle lesions (5g) samples were obtained for isolation (culture) and microbiology. Such samples were labelled, recorded and transported on ice-pack to the laboratory for further analyses.

Investigations of Tuberculosis amongst the Human Patients

Conveniently, all in-patients (n=250) that were suspected to have Tb with or without HIV/AIDS and who reported to the selected hospitals (TB-section) during the period of the study were conveniently sampled and tested. Two general hospitals within the study area were selected for this purpose. An arrangement was made with human doctors in the selected hospitals to help in obtaining ethical clearance (consent) for the sample collections from humans. Full identification of samples via pilot study was made in each case with particular attention paid to the association of the patient with livestock and HIV/AIDS. Sputum (5ml) samples were conveniently collected from such patients and other members of the family with the help of competent medical personnel. Particular attention was paid to the clinical appearance of each family member and his/her medical history where available. All the human sputum samples (250) were collected from Gombe State, no sputum sample was collected from Bauchi State.

Laboratory Analyses

All samples obtained (tissues, lymph nodes, sputum from humans patients in hospitals and those from the family of herdsmen) were transported on ice pack in Cetyl pyridinium Chloride (CPC) to the laboratory for analysis. Ziehl-Nelsen staining for the detection of acid-fast bacteria was performed on all tissues, lymph nodes and sputum collected from the abattoirs and those from the humans respectively, as described by Kazwala *et al*. (1998).

Processing of Tissue Samples and Tissue Culture

Tissue samples were obtained at the selected abattoirs and from the purchased tuberculin positive cattle. All the cattle were necropsied by routine technique as described by Monaghan *et al.* (1994). In cattle with macroscopic lesions, the lesioned material was collected. If no lesion detected, samples were taken from mediastinal, retropharyngeal, and bronchial lymph nodes and from the lung. These samples were numbered and then kept in sterile containers, transported to the laboratory and stored at refrigerating temperature until they were processed. Tissues were divided into equal portions. One-half of each of the samples was kept frozen for confirmatory analysis in case the culture needed to be repeated. All samples were thawed at room temperature and processed as follows. Fat and fibrous tissues were removed aseptically and the surface of each sample was examined for gross lesions. Approximately, 5 g of the remaining tissue was cut into small pieces with a sterile scalpel blade. These pieces of tissues were homogenized with 3ml of sterile distilled water. Two milliliters of the homogenate were separated for decontamination by the addition of 3ml of a solution of 1% sodium hydroxide,3ml of 3% lauryl sulfate, after which they were incubated for 30 minutes. The mixture was then neutralized with 0.5-1ml of 8.75% orthophosphoric acid by using bromocresol blue as an indicator. The neutralized suspension was then centrifuged at 3500rpm for 30 minutes. The supernatant was discarded, and 0.25ml of the pellet was inoculated unto Loewenstein-Jensen (L-J) media with and without pyruvate. The media were checked for growth weekly, for 5 to 8weeks. 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.

Processing of Human Sputa

Sputum samples from suspected patients were processed using the modified Petroff's technique (Sharma *et al.,* 2012). An equal volume of 4 % sodium hydroxide was added to the 5ml of sputum in a universal container. The contents were shaken by hand and incubated at 37^{o}C for 15 minutes, after which the tube was placed in a centrifuge and centrifuged at 3000rpm for 15 minutes. The supernatant fluid was then poured off from the deposit into a 1% sodium hypochlorite solution. The cap was replaced and shaken for a few minutes to re-suspend the deposit. The contents were further centrifuged for 20 mins at 3000r/min. The supernatant fluid was then poured off. The deposit was then inoculated on the entire surface of two Lowenstein-Jensen slopes using a Pasteur pipette at 37^{o}C. The inoculated media were then incubated for 8 weeks in an upright position. Colonies suspected of being *Mycobacteria* were examined for the presence of acid-fast bacilli by Ziehl-Nielsen stain technique.

All acid-fast bacilli positive sample were incubated for further identification and molecular analysis.

Standard Diagnostic(SD) for Tuberculosis Antigen-TB AgMPT64 (SD Bioline^{R})

A commercially available test kit Bioline^{R} (Standard Diagnostic INC, Korea) was procured and used. This test involves a test cassette that consists of a sample pad, a gold conjugate, a nitrocellulose membrane, and an absorbent pad. The immunochemistry-based MPT64 test /Mouse monoclonal anti-MPT64 (*Mycobacterium* protein type-64kDa) was immobilized on the nitrocellulose membrane as the capture material (test line). Other antibodies, which recognized another epitope of MPT64, conjugated with colloidal gold particles were used for antigen capture and detection in a sandwich-type assay. The cassette has a letter T and C as test line and control line on the surface of the case. Positive results produced a red to the purple band. In the absence of MPT64, there is no line in the test region. Briefly, 5-7 colonies were emulsified in about 200µml sterile buffered saline, then, 100µml of the suspension added into the sample well and allowed to stay for 15 minutes before being read, a positive result is indicated by the presence of 2 colour bands (one control band and one test band). The presence of only one control band within the result window indicated a negative result. If the band appeared faint color band, then it was considered as a weak positive or doubtful and the sample was retested.

Molecular Identification

All cultures obtained from samples (necropsy and human sputum) were collected, processed and then subjected to molecular analysis using PCR-based molecular technique; known as Hain assay Test (WHO, 2016). The Hain Assays includes probes to identify MTBC, and detect mutations in the *rpoB* WT3/4 region/gene (associated with rifampicin resistance), as a target gene.

Specimen Preparation

Bacterial culture grown on L-J (Loewenstein-Jensen) media was used to run the Hain assay test. The full loop of bacterial colonies was suspended in 300µl of water (biology grade) and was vortex briefly. The suspension was spun for 15minutes in a standard tabletop centrifuge (Manufacturer TM) with the aerosol-tight rotor in BLS class 3 at approximately 10,000×g to obtain a pellet. The supernatant was then discarded and the pellets were re-suspended in 100-300µl of nuclease-free water by vortexing briefly.

DNA Extraction

Only decontaminated and processed culture specimens were used in this process. The bacterial cells were broken to expose the DNA chemically using the lysis buffer (Genolyse^{R} kits). One hundred microliters (100µl) of distilled water in a 1.5ml screw-capped tube was pipetted. A few colonies (2-3) of *Mycobacterium* Tuberculosis Complex (MBTC) culture on L-J slant were scrapped and were emulsified into distilled water in screw-capped tubes. The tubes were vortexed to mix briefly. Then, 100µl of lysis buffer (A-LYS) was added. The mixture was incubated for 5-8minutes at 95°C in a water bath. The solution was then spun down for 15minutes at 1500g. Then, 5-10µl of the supernatant was used further purified for the DNA to be used for PCR procedure.

Pre-PCR and PCR Amplification

This step dealt with the preparation of Master-mix used for amplification. All reagents needed (polymerases, primers and probe) for the amplification were included in the amplification mixes A and B (AM-A and AM-B). Following the DNA extraction, it was mixed as follows:10µl AM-A, 35µl AM-B, 5µl DNA solution, aliquoted into the PCR reaction tubes up to final volume of 50µl was used for PCR protocol.

All instruments, racks, bench were decontaminated with freshly prepared 0.5% hypochlorite solution before and after loading the thermocycler. The tubes were then loaded onto to thermocycler and the lid closed. On the thermocycler, an appropriate program for the amplification was selected and the procedure was started. The protocol followed the initial denaturation at 95 ^{0}C for 15 minutes, denaturation at 95 ^{0}C for 25 seconds, annealing at 50 ^{0}C for 40 seconds, extension at 70 ^{0}C for 40 seconds and final extension at 70 ^{0}C for 8 minutes. The steps are repeated for 30 cycles. Amplification was determined by hybridization. The visualized band patterns on the strips are then interpreted by either a manual comparison with a printed template or read and analysed in the GenoScan reader (figure 3). The amplification products can be stored at +8 to -20 ^{0}C.

Post PCR Analysis (Hybridization)

A. Denaturation Step

Twenty microliters (20µl) of denaturation solution (DEN) was added into each well of the tray; 20µl of DNA PCR products in each tray mixed by pipetting up and down. Then, it was incubated at room temperature for 1minute (on the bench).

B. Hybridization Step

One milliliter (1ml) of hybridization solution (HYB), containing sodium dodecyl sulfate (SDS) was added to each well containing DEN + amplified DNA mix the solution by tilting the mixture up and down. The strips were labelled according to the sample labelling, using the DNA strip marker. All the labelled strips were placed in each tray corresponding to each sample (the well contains already the mixture DEN, DNA, HYB) and were incubated for 20minutes at 45°c in Twincubator (Fisher Scientific, USA). The whole quantity of HYB was aspirated completely. Then, 1ml of STR (fixing and washing solution) was added to each tray and incubated for 10minutes in the Twincubator. The whole quantity of STR was then removed completely and 1ml of RIN (rinsing solution) was added to each tray and was incubated for 1minute at room temperature in the Twincubator. The whole quantity of RIN was then removed.

C. Detection

One milliliter (1ml) of the diluted conjugate was added and incubated for 20 minutes at 37°C in the Twincubator (for dilution:10µl conc-C+ 990µl conc-D). The conjugate was then aspirated completely.1ml of RIN (rinsing solution) was

added to each tray and incubated for 1minute at room temperature in the Twincubator. The whole quantity of the RIN was aspirated completely, 1ml of the substrate, dilution (10µl SUB-C + 990µl SUB-D), was added to each tray and incubated for 2-10 minutes at room temperature in the Twincubator. The substrate was then aspirated completely. The reaction was stopped by rinsing twice with water for 1 minute in each rinse.

Mounting and Interpretation:

The DNA strips were removed from the tray and air-dried on an absorbent paper. All the dried strips were then pasted to result in sheet for interpretation according to the manufacturer's laboratory manual (Hain Lifescience, Germany).

Data Presentation and Analyses

Data were presented in the form of Tables, Bar charts, graph, and figures, while analysis was done to estimate the prevalence of bTB in the study area. 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.

Data Analysis

Descriptive statistics were carried out using Microsoft Excel (Version 2016 for Windows 10), and imported into statistical package for social sciences (SPSS^R Version 20.0 for Windows^R, SPSS Inc., Chicago, USA). Chi-squared (χ^2) test of association was calculated, to determine the possible association between age, sex and season with the tuberculosis in cattle. Odds ratio (OR) and 95 % confidence interval (95% CI) were determined to estimate the risk. The allowable error value of P< 0.05 was considered statistically significant throughout the study.

RESULTS

Bovine Tissues from Abattoir

A total of 100 tissue samples each from Bauchi and Gombe States were collected from carcasses during post-mortem examinations from the designated abattoirs (Table 1). Forty-five (45 %) of the samples from Bauchi was from male carcasses while the remaining 55 % were female, similarly, 35 % of the carcasses from Gombe State were male and the remaining 65% were 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 5 years old (Table 1). There was no significant association between the positive samples obtained and the age group as well as that of sex of the animals and positive samples for TB (P>0.05) (Table 1).

Table 1: Age and Sex Distribution of Tuberculous Lesions among Slaughtered Cattle in Bauchi and Gombe States Abattoirs, and their Level of Associations

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)			1 (Ref)
> 5	165	25 (15.2)	4.87	0.0000	11.8 (5.20-26.73)
Total	**200**	**35 (17.5)**			
Sex					
Male	80	18(9.0)			1 (Ref)
Female	120	34(17.0)	10.7	0.0011	2.83 (1.50-5.36)
Total	**200**	**52(26.0)**			

Prevalence of Bovine Tuberculosis in Bauchi and Gombe States (Based on Organ Distribution).

Based on the post-mortem 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. The 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 LN with 25.0 %, submandibular lymph had 23 % in Bauchi and 12.5 % in Gombe, while the least was seen in others (heart, intestines, and liver) with 6.5 % and 8.20 % for Bauchi and Gombe respectively.

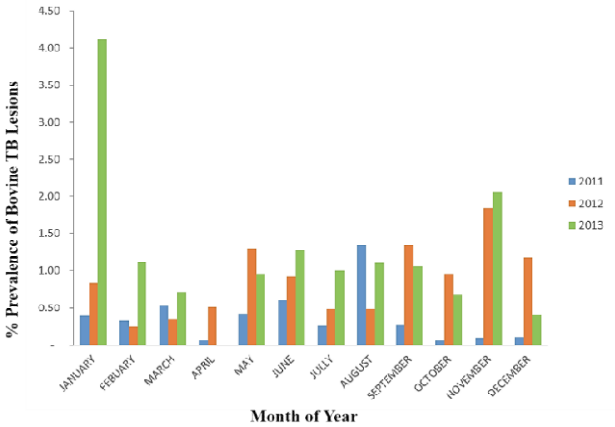

Figure 1: Monthly Distribution of TB-lesions observed at the Gombe Abattoir between the period of 2011-2013, indicating the number of TB-lesions collected and the sequence of occurrence for the number of animals slaughtered during the year 2011-2013.

The monthly prevalence of suspected Tb lesions at the abattoirs under study are presented in Figure 1, indicating the number of various lesions collected and the sequence of occurrence for the number of animals slaughtered and lesions observed during the year observed against time is presented in Figure 1.

The lymph nodes were mostly caseous while most lesions in the lungs were observed at the parenchyma, due to high oxygen tension. From the study, it was observed that the seasonal index for lesions in the abattoirs in Gombe State had peaked in February and July with lesser peaks in April and November (Figure 2).

Culture and Isolation of Mycobacteria from Bovine Tissues from the abattoirs and Cattle Positive by Caudal-Fold Test (CFT).

Of the 200 samples collected and cultured, 138 (67 %) were negative or had no growth on them after 8 weeks of incubation, while 62 (32.5 %) were positive based on cultural characteristics and the Zeihl-Neelsen (ZN) Stain technique. Approximately, 25(12.5 %) of the culture-positive samples were found to be contaminated (Table 3). This analysis yielded 18 as MTBC, while the remaining seven were characterized as Non-Tuberculous Mycobacteria (NTM) (Table 4). Of the 62 positive samples by AFB, 18 (9.0 %) were found to be positive for MTBC following subjection to Bioline analysis, the remaining were characterized as NTM (Table 4).

Culture and Isolation of Mycobacteria from Human Patients Sputa

Two hundred and twenty-five (250) smear positives sputum were collected from Gombe State. No sputum samples could be collected from Bauchi State. All the sputum samples collected were then cultured on solid media (Lowein-Stein-Jensen) media supplemented with glycerol and pyruvate.

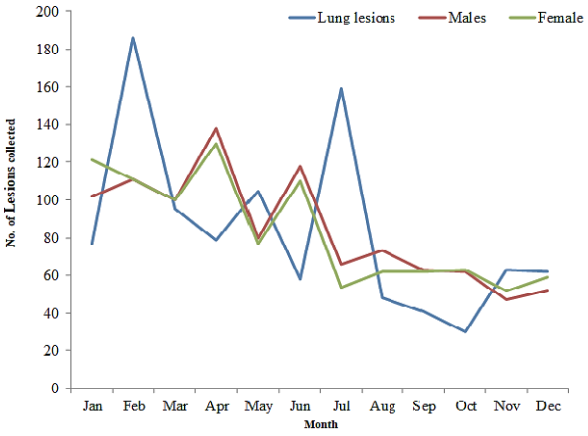

Figure 2: Sequence of occurrence of number of animals slaughtered and lesion observed during the years (2011-2014).

Table 2: Summary of Slaughter House Survey for Suspected TB Lesion in Bauchi and Gombe States (2011-2014)

State	Location	No. Cattle slaughtered	No. cattle 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 3: Organ Level Distribution of Suspected Bovine Tuberculosis Lesions found during Abattoir PM-Inspection

Organs inspected	No. of Tb lesion	Percentage (%) of Organ Specific TB lesion
Bauchi (n = 5671)		
Lung	1985	35.0
Mesenteric 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
Mesenteric LN	1703	21.0
Mediasternal LN	1334	16.44
Submandibular LN	983	12.12
Tracheobronchial LN	942	11.61
Others (heart intestine, liver etc)	665	8.2

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

Type of specimen	No. of samples collected	AFB Positive (%)	Culture Positive (%)	Bioline Positive (%)
Sputum	250	105(42)	74(29.6)	40(16)
Tissue	200	62(32.5)	25(12.5)	18(9.0)

on cultural characteristics and the ZN staining technique. Furthermore, 98(39.2 %) were either contaminated or the culture had dried-up, while 78 (31.2 %) were negative or had no growth on them after 8 weeks of incubation (Table 4). The 74 cultured and smear-positive samples on being subjected to Bioline ® (SD TB AgMPT 64 rapid) analysis showed 40 (16.0 %) were classified as MTBC (Table 4), and the remaining 34 (13.6 %) were NTM.

Hain Assay PCR Analysis

All positive isolates on Bioline® analyses were further subjected to Genotype® MBTC analysis. All sputum and tissue isolates identified as MBTC were further subjected to Genotype MBTC® Hain assay PCR analysis in order to differentiate them into their various species. Of the 18.0 MBTC isolates from the tissues, 8(4.0 %) were found to be *Mycobacterium bovis,* 5(2.5 %) were *Mycobacterium tuberculosis* and the remaining 5(2.5 %) were unidentified using the standard banding patterns. The 40 MBTC from sputum showed 30 (75 %) as *Mycobacterium tuberculosis,* 6(15 %) were *Mycobacterium bovis* and 3(7.5 %) were *Mycobacterium africanum* and 1(0.4 %) was unidentified using the standard banding patterns (Figure 3).

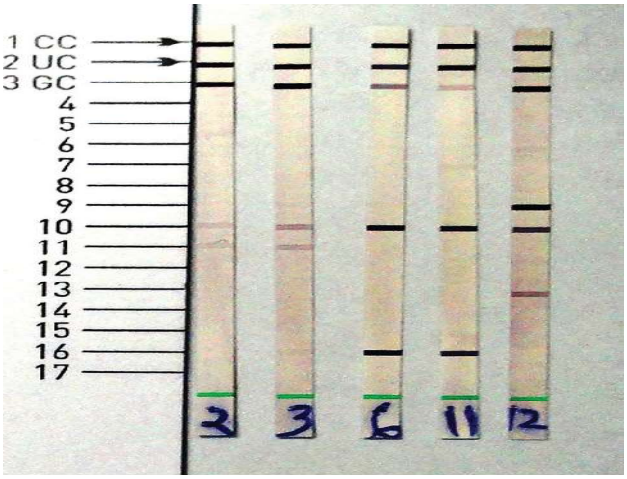

Figure 3: Results showing the standard banding patterns on the strips in Hain Assay PCR, indicating the *Mycobacterium tuberculosis, M. bovis* and *M. africanum* in the isolates.

DISCUSSION

In 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. The high prevalence of Tb found in this study is in agreement with previous reports by Aliyu *et al*. (2009) who reported 12.27 % in cattle in Gombe, while Saidu *et al. (*2015) reported an abattoir prevalence level of 8.8 % in cattle in Bauchi State. Similarly, a prevalence rate of 8.30 % was reported by Cadmus *et al*. (2004) in in cattle in Ibadan, Nigeria. The prevalence rate of bovine tuberculosis found in this study was higher than the earlier reports of bovine tuberculosis in other parts of Nigeria. For example, Aliyu *et al.* (2009) had reported a relatively lower prevalence rate of 0.34 % in Adamawa State, while in Ogbomosho, Osun State, Ameen *et al.* (2008) reported a prevalence of 0.55 % and Nwanta *et al.* (2011) had reported a prevalence of 1.4 % in Enugu State. Similarly, researchers in other parts of Africa reported a relatively lower prevalence rate of bovine Tb in cattle. For instance, in Cameroon, 6 % prevalence rate was reported by Thoen, 2009, and Milan *et al.* (2000) also reported a prevalence of 9 % in Mexico. The difference in the prevalence rate might be due to different locations and methods used in their studies. However, prevalence of tuberculosis lesions recorded in this study in slaughtered cattle in Gombe and Bauchi abattoirs may be due to the fact that there is no active bovine tuberculosis control program in Nigeria and that movement of cattle across both local and international boundaries was not restricted. These porous borders may promote the entrance of infected animals from neighbouring countries such as Chad, Niger Republic, and Cameroun as suggested by Aliyu *et al.* (2006). Another explanation for the high prevalence of tubercle lesions in cattle slaughtered in Gombe and Bauchi States could be due to the absence of antemortem examinations and consequent poor veterinary and public health system. During the late dry season and early rainy season, the prevalence of tubercle lesions was 12.89 % in Gombe and 10.95 % in Bauchi while the prevalence rate was lower during the late rainy season (7.6 %) in Gombe and 6.9 % during early dry season in Bauchi. These findings were different from that of Awah-NduKum *et al.* (2010) in Cameroun who reported that the detection of TB like lesions was not influenced by season but high when the cattle were stressed and immunocompromised such as during inter-season and peak periods. Similarly, Ameen *et al*. (2008) reported similar findings in Ogbomosho, Nigeria. However, Bikom and Oboegbulum (2007) reported a strong association between season and tuberculous lesions but the reason for the difference in the seasonal variations observed in their study was not clearly stated.

The findings of this study showed that there was no association between sex and tuberculous lesions. These findings agreed with that of Nwanta *et al.* (2011) and Awah-Ndukum *et al. (*2010) who also reported that, there was no association between sex and tuberculous lesions detected in the abattoirs. In this study, the organs found to have suspected Tb lesions appeared to be similar to those reported by other researchers; in that the highest lesions appeared to be in the lungs and could be due to the fact that, the animals acquired their infections through aerosols during grazing or at night. It could also be through the addition of new animals to the herds as explained by Kaltungo *et al.*

(2013), who reported that pastoralists commonly adding new animals into their herds without any quarantine. Furthermore, our findings also agreed with that of Ameni and Woldie (2003) who showed that 72 % of the gross lesions were in the thoracic cavity, while Regassa *et al.* (2009) and Tigre *et al.* (2011) also detected 50 % and 48.4 % of Tb lesions respectively in the respiratory pathways of cattle.

The study has shown a high prevalence of Non-tuberculous Mycobacteria (NTM) otherwise known as environmental *Mycobacteria* in the cattle studied to be 7.5 % and 13.6 % in humans. This signifies environmental contamination might have a role in the study area. Cattle could have acquired the infection through a contaminated environment during grazing or at water sources. The prevalence of NTM seen in this study in both cattle and humans is lower than the previous report in Jos, Nigeria by Mwak *et al.* (2006) who reported a prevalence of 23.08 % in humans. Similarly, Shirima *et al.* (2003) and Durnez *et al.* (2009) in Tanzania reported prevalence of 2.5% and 10.1% for *M. bovis* infection and atypical mycobacterioses respectively in cattle. Furthermore, Mdegela*et al.* (2004) reported a prevalence of 14 % and 19 % of NTM in cattle (milk samples) in Kibaha and Morogoro, respectively. Although this study did not investigate milk and its products, it is a known practice in the study area that consumption of unpasteurized milk is a very common practice and this might expose milk consumers in the study area to a greater risk of infection. The prevalence of NTMs as obtained in this study might have predisposed some of them to infection with HIV/AIDS. Similarly, Gumel *et al.* (2015) reported a high prevalence of NTM in HIV/AIDS patients in Zaria Kaduna State, Nigeria.

The result of this study indicated that *M. bovis* and *M. tuberculosis* were identified in slaughtered cattle in the study area, indicating an important finding with economic and public health consequences. Thus, humans, especially the pastoralists who were in a habit of consuming fresh and soured milk were at high risk of coming down with bTB. This study has also shown that cattle come down with human-based TB since *M tuberculosis* was recovered from them (an indication of reversed zoonoses). Thus, the habit of pastoralists living closely with their cattle at night could account for this. Similarly, the finding of *M tuberculosis* in human sputum could be either through human to human or even animal to human infection since some of the cattle were found to harbour *M tuberculosis*. For the same reason, the finding of *M. bovis* in human sputum could be from cohabitation with the cattle in their herds (a zoonotic linkage). A study by Byrugaba *et al.* (2009) reported that *M. bovis* was responsible for 3.1 % of all forms of human TB worldwide. Other studies previously have claimed the prevalence of *M. tuberculosis* in cattle to have always not exceeded 1 % (Ocepek *et al.,* 2005) but findings from this study, found the prevalence to be higher 9.0 %. 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 (Romero *et al.,* 2011). The isolation of *M. bovis* and *M. tuberculosis* in slaughtered cattle confirm that there is zoonotic tuberculosis in the study areas, which should be considered a public health concern especially among the pastoral communities that have strong attachments to their cattle for cultural, social and economic reasons (Oloya *et al.,* 2007). In this study, the majority of MTBC cases were caused by *M. tuberculosis,M. bovis* and *M. africanum* in the human sputum samples analysed. The proportion of *M. africanum*is low compared to previously reported studies done in some parts of Nigeria. For instance, Cadmus *et al.* (2006) reported 13% in Southern Nigeria, while Waziri *et al*. (2014) reported 10.8 % in pulmonary patients in Zaria, Kaduna State. The reason for the low prevalence of *M africanum* in this study could be due to the different techniques used. The finding of *M. africanum* in the milk of pastoral cattle in Nigeria by Cadmus *et al.* (2010) could account for the identification of this organism in the sputum of the pastoralists as found in this study.

Conclusion

The study revealed a higher prevalence of bTB due to MTBC (*Mycobacterium tuberculosis, M. bovis and M. africanum*); 9.8 % in Bauchi and 13.9 % in Gombe, signifying the public health importance of bTB in the study area as endemic and zoonotic one. The study also revealed a significant number (13.6 %) of NTM/ atypical *Mycobacterium* species. There indicates the need for public health awareness programs on the zoonotic nature of bTB particularly among the abattoir workers and the herdsmen. Majority of the MTBC isolates from the human sputum samples were due to *M. tuberculosis, M. bovis* and *M. africanum.* as 30 (75 %), 6(15 %) and 3(7.5 %) respectively. However, only 1(0.4 %) was unidentified using the standard molecular banding patterns.

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Authors' Contribution

Field work, sample collection and transportation of samples to the Laboratory was done by Ibrahim S, Laboratory analysis of sample was performed by Danbirni S, U.B Abubakar and Saidu AS. The first draft of the manuscript was written by Ibrahim S and Mohammed F.U and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors have no conflict of interest to declare.

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