British Journal of Medicine & Medical Research
5(10): 1220-1229, 2015, Article no.BJMMR.2015.138
ISSN: 2231-0614

SCIENCEDOMAIN *international*
www.sciencedomain.org

Molecular Identification of *Mycobacterium bovis* from Post-mortem Inspected Cattle at the Abattoir and Slaughter Houses in Bauchi State, Nigeria

A. S. Sa'idu[1*], E. C. Okolocha[1], A. A. Dzikwi[1], J. K. P. Kwaga[1], A. Usman[2] A. A. Gamawa[3], U. B. Abubakar[4] and S. A. Maigari[5]

[1]Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, P.M.B. 1013, Zaria, Kaduna State, Nigeria.
[2]Tuberculosis Laboratory, Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, P.M.B. 1013, Zaria, Kaduna State, Nigeria.
[3]College of Agriculture, Ministry of Higher Education, Bauchi State, Nigeria.
[4]Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, P.M.B.1013, Zaria, Kaduna State, Nigeria.
[5]Department of Obstetrics and Gyenecology, University of Maiduguri Teaching Hospital, Maiduguri, Borno State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author ASS designed the study, did the sampling, run the Ziehl-Neelsen staining technique and wrote the first draft of the manuscript. Authors ECO, AAD and JKPK supervised the whole research and reviewed the manuscript. Authors AU and UBA managed the molecular aspect and provided the primers and positive controls used in the PCR. Author AAG contributed in the data collation and managed the data analyses of the study. Author SAM did the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2015/11650
Editor(s):
(1) Jimmy T. Efird, Department of Public Health, Epidemiology and Outcomes Research and East Carolina Heart Institute, Brody School of Medicine, Greenville, North Carolina, USA.
Reviewers:
(1) Travería Gabriel Eduardo, Veterinary Science Department, La Plata University, Argentina.
(2) Anonymous, University of Zambia, Zambia.
(3) Anonymous, University College Dublin (UCD), Ireland.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=717&id=12&aid=6730

Original Research Article

Received 27th May 2014
Accepted 18th August 2014
Published 30th October 2014

ABSTRACT

Aims: Bovine *tuberculosis* (bTB) is a chronic infectious and contagious zoonotic disease of domestic, wild animals and humans. The disease occurs in a wide range of mammalian species and therefore, poses a public health threat. It also results in considerable economic losses in

**Corresponding author: Email: adamudvm13@gmail.com;*

livestock production and carcass condemnation of infected cattle during meat inspection. This study was aimed at determining the prevalence of zoonotic bovine *tuberculosis* in slaughtered cattle, based on Post-Mortem (PM) meat inspection, Ziehl-Neelsen staining (ZN) and Polymerase Chain Reaction (PCR) techniques in abattoir and slaughter houses in Bauchi State, Nigeria.

Place and Duration of Study: A cross-sectional abattoir based-study was conducted on 800 slaughtered cattle in the Northern, Central and Southern Zonal abattoirs of Bauchi State, Nigeria. This work was carried out between June-September, 2013.

Study Design: Experimental.

Methodology: One hundred and twenty (120) tissue samples from different organs were suspected to have bTB lesions at PM 15% (120/800). Out of the samples examined 35 (29.2%) were Acid-Fast Bacilli (AFB) positive; 10 (8.3%) of which were confirmed positive for *M. bovis* by the confirmatory Polymerase Chain Reaction (PCR).

Results: The present study found the prevalence rates of 3.33% (4/120) and 5.00% (6/120) for males and females, respectively. This gave an overall prevalence of 8.33% for bTB (*M. bovis*) based on PCR. Bovine TB sex-specific rates were 10.00% (4/40) and 7.50% (6/80) by PCR, in males and females respectively. Female cattle also had a higher prevalence than male cattle but there was no statistically significant association ($p>0.05$, $x^2 = 0.218$) between the presence of bTB in the tissues sampled and the sex of the cattle. There was a statistically significant association ($p<0.05$, $x^2 = 7.002$, OR=3.363) between detection of bTB in suspected tissues and the age of cattle. Using ZN, cattle aged six (6) years and above had the highest number of positive bTB cases 67.9% (31), while cattle aged 3-5 years old had the lowest 14.81% (4/27). PCR technique, revealed age-specific prevalence rate in cattle aged 6-8 and 9-11 years were 17.07% and 5.77%, respectively. Bauchi zonal abattoir had the highest number of suspected bTB cases (62.5%), followed by Katagum (26.7%) and Misau (10.8%).

Conclusion: High infection rate of bTB was found among cattle sampled in the study area, with a significant prevalence in Bauchi metropolitan abattoir than the other two (2) slaughter houses (Katagum and Misau). This showed that the prevalence of bTB was higher in Bauchi metropolitan abattoir which supplies larger population of the state with beef. These findings also demonstrated that, there is urgent need for public health authorities in the state to intervene.

Keywords: Abattoirs; Bauchi; cattle; Mycobacterium bovis; prevalence; post-mortem; Polymerase Chain Reaction (PCR).

1. INTRODUCTION

Bovine *tuberculosis* is a chronic infectious and contagious zoonotic disease of domestic animals, wild animals and humans [1]. It also occurs in a wide range of mammalian species [2]. It is characterized by granulomas in tissues especially in the lungs, lymph nodes, liver, intestines and kidney [3]. *Tuberculosis* is a major health problem with 8 – 9 million new cases and 3 million deaths annually worldwide [4]. The majority of these occur in the developing nations [5]. In Nigeria, there have been limited studies to determine the prevalence/relationship between bovine and human TB especially with the emerging culture of eating improperly cooked beef and mutton, along with the drinking of unpasteurized fresh milk [6,7]. The authors [8] also reported an economic loss of 13, 871,014/annum (US 86, 693.84) with associated public health implications due to *tuberculosis* as major reasons for condemnations in some abattoirs in Western-Nigeria.

Bovine *tuberculosis* is caused by *Mycobacterium bovis* which is a member of *Mycobacterium tuberculosis* complex [9,10]. The aetiological agents of mammalian tuberculosis, classified as members of the *Mycobacterium tuberculosis* complex (MTBC), include: *Mycobacterium tuberculosis, M. bovis, M. microti, M. caprae, M. africanum, M. canettii, and M. pinnipedii*. *Mycobacterium africanum* consists of a rather heterogeneous group of strains isolated from humans in Africa [11]. *Mycobacterium bovis*, otherwise known as the bovine tubercle bacillus is the cause of bovine tuberculosis, the organism may be transmitted by aerosol, by ingestion of feed and water contaminated with urine, fecal material or exudates from diseased animals that contain the tubercle bacilli [12]. The bovine tubercle bacilli is usually assigned to bTB in cattle, but still is often used to denote bovine strains of the tubercle bacillus irrespective of the host. In fact, the bovine tubercle bacillus has one of the broadest host ranges of all known pathogens. The species has been reported in

domesticated and feral bovidae. Other species reported include goat, sheep, pig, horse, cat, dog, fennac fox, bison, buffalo, badger, wild and feral pig, antelope, camel, man and primates, among others [13]. Cattle movements, particularly from areas where bTB was reported, are the best predictor of disease occurrence [14].

The genus *Mycobacterium* is the only genus in the family *Mycobacteriaceae*. The high moles percent G + C content of the DNA of *Mycobacterium* species (62–70%) is similar to that of the other mycolic-acid - producing bacteria, *Norcardia asteroides* (60–69%), Rhodococcus (59–69%) and *Corynebacterium* (51–59%), thus molecular identification by polymerase chain reaction (PCR) or other molecular techniques can be adopted as a confirmatory diagnostic means of *Mycobacterium* species [12].

Mycobacterium bovis is a zoonotic member that possess a unique genomic characteristics that distinguish it from other members of MTBC; known as an *Oxy*R gene. *Oxy*R gene is a regulatory protein that functions as both an oxidative-stress sensor and activator of gene transcription. The 285bp *oxyR gene* amplicon which is located within 16-23 rRNA regions and is known to be part of the *Ahp*C- *oxy*R regulon at position 285bp Guanidine-nucleotide sequence of the *M. bovis* genome (species-specific for *M. bovis*), coding for INH (Isoniazide) susceptibility and resistance of the organism, as it plays a role in the Hydrogene peroxide reductase system, regulating the endogenously generated hydrogen peroxide in the bacteria [15].

Bovine TB in cattle takes as long as a few years before any signs can be observed. As the infection progresses in cattle, bovine *tuberculosis* shows a slight fever, roughened hair coat and the lymph nodes usually swell or have abscesses and show signs of dyspnoea with an associated cough. The cow will appear weak, and show signs of laboured breathing and have an increased breathing rate. Anorexia will be noticed and decrease in weight may be so significant that the cow actually becomes emaciated [15].

Tuberculosis remains a major public health problem worldwide [16,17]. Factors such as inadequate health service infrastructure, decreased access to health care and limited human and financial resources have prevented adequate implementation of control measures against TB [18]. Recent advances in the treatment of human TB has permitted effective management of cases on an ambulatory basis [19]. However, in many developing countries, an irregular drug taking practice and premature termination of treatment mainly by self-discharge is the main cause of poor perfomance of control programmes.

Inhalation is the major route of transmission, hence respiratory signs, clinical findings like Progressive emaciation, capricious appetite, and fluctuating temperature with signs referable to localization such as respiratory disease, pharyngeal obstruction, reproductive disorder, and mastitis are diagnostics. In pigs the disease is subclinical but tuberculous lesions in cervical lymph nodes, Clinical pathology and tuberculin testing are also diagnostics. Single intradermal test is the official test in most countries with the comparative test for cattle inspected as false-positive reactors. Interferon-gamma test, Necropsy findings of tuberculous granulomas may be found in any of the lymph nodes, or there may be generalized [1].

The organism has been, until very recently, dependent on laborious determinations of cultural characteristics and susceptibility to therapeutic agents. Exact definition of mycobacteria is now possible by nucleic acid probes and molecular techniques [1].

This study was aimed at determining the prevalence of zoonotic bovine *tuberculosis* in slaughtered cattle, based on Post-Mortem (PM) meat inspection, Ziehl-Neelsen staining (ZN) and Polymerase Chain Reaction (PCR) techniques.

2. MATERIALS AND METHODS

2.1 Study Area

Bauchi state occupies a total land area of 49,119 KM² representing about 5.3% of Nigeria's total land mass and is located between latitudes 9° 3' and 12° 3' north of the equator and longitudes 8° 50' and 11° east of the Greenwich Meridean [20]. The State is bordered by Kano and Jigawa to the north, Taraba and Plateau to the south, Gombe and Yobe to the east and Kaduna to the west. The state is highly populated with cattle mainly owned by Fulani herdmen. The cattle population is estimated at 1,789,000; about 13% of the Nigerian cattle population of 13,900,000 [21]. The State has a human population of 4,676, 465 (3.34%); ranked 11th of the 36 states, density of 95km^2 (250/sqmi) and per capita income of

983 [22]. Bauchi State has a total of 55 tribal groups in which Hausa, Fulani, and Kanuri are the main tribes. The study was carried out in Bauchi, Katagum and Misau Local Government Areas (out of the 20 LGAs), each representing a senatorial zone as Bauchi South, Bauchi North and Bauchi Central with populations of 493,810, 295,970 and 263,487 respectively.

2.2 Sampling Size Determination

- As described by Mugo [23]; $N = Z^2 PQ / d^2$

Where:

N = sample size
Z = value for the corresponding confidence level (e.g 1.96 for 95% confidence interval)
P = estimated value for the proportion of a sample/expected prevalence
Q =1-P, d= is the margin of error (e.g 1= ± 10%).

$$N = \frac{1.96^2 \times 0.0172\ (1-0.0172)}{0.01^2} = 730$$

- N≈800

A sample size of 730 cattle was estimated from the expected prevalence rate of 1.72% [24] as described by [23]. However, 800 slaughtered cattle were examined for bTB lesions from the three (3) selected LGA abattoirs and slaughter houses.

2.3 Sampling Procedure

The Bauchi abattoir and slaughter houses of Misau, and Katagum (Azare) each representing one of the senatorial zones of Bauchi State was included in this study for sampling purposes. Identification of cases was based on presence of typical tubercle, yellowish, granulomatous and caseous lesions in the lungs, lymph nodes, kidneys, intestines, and liver [25]. Aging of cattle was carried out at the abattoir after slaughter as described by [26]; using the time of appearance and the degree of wear on the temporary and permanent teeth. Additional data taken included sex and breed of each animal sampled.

A total of 800 slaughtered cattle were examined at post-mortem for TB-lesions. Using a convenient sampling; a total of 400 cattle from Bauchi abattoir, 200 cattle each from Misau and Katagum LGA slaughter houses were examined. One hundred and twenty (120) tissue samples were collected from cattle with suspected TB-Lesions into sterile screw – capped containers (with 9% normal saline solution to keep them moist) and transported inside a cooler containing ice pack to the Veterinary Public Health Bacterial Zoonoses Laboratory, Ahmadu Bello University, Zaria and stored at -20°C until analysis was carried out.Stored samples were processed (crushed and analysed) for microscopic examination (Ziehl-Neelsen staining technique) and only AFB positive samples were taken to the DNA- LABS, Kaduna (to reduce the cost implication associated with running the 120 samples), and used for conventional PCR amplification protocols specific for *M. bovis*, as described by [27].

2.4 Genomic DNA Preparation from Tissues

The tissue samples positive by ZN were collected into a 1.5ml microfuge tube containing lysis buffer, stored at room temperature and transported on ice pack to DNA-LABS, Kaduna. About 1g of the tissue sample was homogenized with pestle and mortar for chromosomal DNA extraction using a phenol-chloroform technique as described by [27,28]. The supernatant was discarded; the suspension was then cooled at 4°C, neutralized with 3 volumes of 0.1 m Tris-HCL (PH. 7.4) buffer, and centrifuged (5,000 × g, 5 min) to get rid of the tissues' membrane and possible contaminants. The pellet was dried and re-dissolved in 20µl of 1xTE buffer and DNA was precipitated with ethanol, collected by centrifugation and dissolved in 50µl of distilled water. Five (5µl) of the extracted DNA was run on 1.0% agarose gel and spectrophotometer to confirm the presence of DNA. The remaining DNA samples were stored at -20°C until further use.

2.5 PCR Amplification Protocols

Ten (10) microlitres suspended DNA was used as a template for PCR amplification under standard conditions as described by [29]. A commercial "Hot-Stat" PCR Premix (Bioneer, USA), a mixture prepared in a lyophilized format containing: Taq DNA polymerase, Reaction buffer, dNTPs (dATP, dGTP, dCTP, dTTP) and $MgCl_2$ was used. All amplification reactions were performed using a Perkin Elmer Thermocycler (Perkin Elmer Cetus) programmed for 40 amplification cycles (Rodriguez, 1995). The reaction was performed in a final volume of 50µl containing 10µl of DNA Template, 1x TE reaction buffer [containing

10mM Tris-HCl (pH 8.3), 50 mM KCl, 1.3 mM $MgCl_2$, and 0.001% of gelatin], 2.5U Taq polymerase, 0.2 mM of each deoxynucleoside triphosphate, and 75 pmol of each primer (Table 1). DNA from *M. bovis* ATCC 19210 and BCG Pasteur 27291 and sterile nuclease-free water were used as positive and negative PCR controls, respectively. After an initial denaturation step (at 94°C, for 5 min.), 40 amplification cycles were performed as follows: Denaturation at 94°C for 1minute, annealing at 58°C for 30seconds and extension at 72°C for 30seconds; with an increment of 1second per cycle for the denaturation and extension segment. A final extension was performed at 72°C for 15 minutes.

2.6 Gel Electrophoresis

After amplification, PCR product was loaded in a 1% agarose gel (Swekem; FMC Bioproducts, Rockland, Maine, USA) containing 0.5pug/ml ethidium bromide. The gel was also loaded with the 100 base pair (bp) DNA molecular marker (Ladder). Gel electrophoresis was done at a 5v/cm for 1 hour. Finally, after electrophoresis, the DNA bands were visualized (using UV light box or gel imaging system) and photographed.

2.7 Data Analysis

The prevalence of bovine *tuberculosis* was calculated using the formula:

$$\text{Prevalence} = \frac{\text{umber of samples positive}}{\text{Total samples analysed}} \times 100(\%)$$

Data were analysed using statistical package for social sciences (SPSS) version 20.0. Chi – square (x^2) was used to determine possible association of age, sex and location with *M. bovis*. Odds Ratio (OR) and 95% confidence interval were calculated to measure the strengths of associations between variables and bTB (*M. bovis*). Tables and bar charts were constructed using Microsoft Excel(R) 2010. Values of $P<0.05$ were considered significant.

3. RESULTS

The present study estimated the prevalence rate of bTB in Bauchi State at 15.0%, 29.16% and 8.33%, based on PM, ZN and PCR, respectively. Cattle aged 6-8 and 9-11years had a prevalence rate of 17.07% and 5.77%, respectively, while cattle aged 3-5 years were negative for bTB (*M. bovis*) using PCR (Table 1). Furthermore, there was no statistically significant association ($p>0.05$; $x^2=2.017$, OR=1.80) between the presence of bTB in the tissues sampled and the sex of the cattle. The male and female cattle sampled had prevalence rate of 37.50% and 25.00%, respectively. However, the male also had a higher sex-specific rate 10% (4/40) using PCR technique than the female cattle 7.50% (6/80) (Tables 2 and 3).

The prevalence rates of the population indicated that 3.33% (4/120) of the 40 male cattle sampled 10.0% (4/40) and 5.00% (6/120) of the 80 female cattle sampled 7.50% (6/80) were also positive for bTB (*M. bovis*) by the confirmatory PCR. The infection rates were higher in females than in males, with the overall prevalence rate of 8.33% for bTB (*M. bovis*) in Bauchi State, and there was no statistically significant association between detection of bTB in tissues and sex of animals sampled (x^2 = 0.218, df=1, OR=1.37, 95% CI on OR=0.364-5.164) (Table 2). However, on chi-square analysis at 95% confidence limit, the ZN and PCR specificity and sensitivity and association for detecting the bTB, showed a high infection rate of bTB in cattle by both diagnostic tests and there was a statistically significant differences between the two tests' sensitivity and specificity found in this study ($p<0.05$, $x^2=17.09$, OR= 4.53, df =1, and 95% CI on OR= 2.123-9.664) (Table 3).

Plate 1: is an agarose gel electrophoresis of PCR products using DNA samples of *Mycobacterium bovis.* Among the 120 tissue samples from affected cattle by PM, only 10 (8.33%) were positive for *M. bovis* by PCR, amplifying the 285bp fragments of the *oxy*R gene.

4. DISCUSSION

The prevalence rates of bTB found in this present study based on ZN and confirmatory PCR were 29.16% and 8.33%, respectively. This study also showed that PCR is a highly sensitive and specific (8.33% and 90.1% respectively) test that can be adapted as a confirmatory test to conventional tests such as PM, TST and ZN because of its ability to rule out possible false positive results associated with these tests. However, ZN has the ability to detect more AFB positive samples as fastidious organisms that may be present in the granulomatous lesions obtained from slaughtered cattle.

Table 1. Specie-specific primers for *Mycobacterium bovis* used for the study

Primer direction	PCR primer sequence	Amplicon size (bp)
Forward	(5'CCCGCTGATGCAAGTGCC3')	285bp *M. bovis*
Reverse	(5'CCCGCACATCCCAACACC 3')	Source: [30]

These primer oligonucleotide sequences were used to amplify the oxyR amplicon [29]

Table 2. Overall age and sex-specific Prevalence rates of bTB (*M. bovis*) among various age groups of cattle (by PCR) in Bauchi State, Nigeria

		No. sampled	PCR positive (%)	Prevalenc (%)	OR	OR (95% CI)
Age	3-5	27	0 (0.00)	0.00	1	
	6-8	41	7 (17.07)	5.83	*3.363	0.812-13.930
	9-11	52	3 (5.77)	2.50	2.331	0.688-7.892
Sex	Male	40	4(10.00)	3.33	1.37	0.364- 5.164
	Female	80	6 (7.50)	5.00	1	
Total		120	10	8.30		

**Significant at 95%CI; Age: x^2=7.002, df=2, p<0.05; Sex: x^2=0.218, df=1, p<0.05*

Table 3. Overall sex-specific and prevalence rates of bTB (by ZN and PCR) in Bauchi State, Nigeria

Sex	Tests			Overall prevalence (%)	
		ZN	PCR	*ZN	*PCR
	No. sampled	Positive (%)	Positive (%)		
Males	40	15(37.5)	4(10.00)	12.5	3.33
Females	80	20(25.0)	6(7.50)	16.66	5.00
Total	120	35	10	29.16	8.33

**Significant diffrences between the two tests),*x^2=17.09, df=1, OR=4.53, P<0.05, 95%CI=2.12-9.664*

This agreed with the previous report by [31] using ZN method alone. The overall prevalence rate (8.33%) of this study is relatively high and may be related to the poor control measures in the state (lack of test and slaughter policy, absence of control at borders, inadequate quarantine measures, and the lack of effective preventive measures against bovine TB) and the influx of possibly infected cattle from neighbouring states and countries (Cameroon, Chad and Niger). Also due to the increase in intensive farming practice where large herds are housed together for long periods of time and poor hygiene are possible contributory factors to the spread and the endemic nature of the disease in the state. There are many studies on bTB in Nigeria and other countries Aliyu et al. [24]. A retrospective study by Aliyu et al. [24] reported a relatively lower prevalence of 1.72% in Bauchi State as compared to our findings, which may be due to the improved diagnostic technique used in this study. Moreover, increase in free influx of possibly infected cattle from neighbouring endemic states, especially, Gombe State with a prevalence rate of 12.27% as reported by Aliyu et al. [24] who described Bauchi State as a population at risk, could also be a reason for the high prevalence reported in this study. Also a high prevalence rate of the disease in the Northern Nigeria, and increase in livestock density and contact rates among cattle of different sources could be another reason.

The findings that, 120 (15%) bTB suspected lesions were observed in the 800 slaughtered cattle examined in Bauchi State abattoirs had emphasized the importance of PM meat inspection. This agrees with the report of [32], as post-mortem examination still remains the immediate diagnostic tool to be used in endemic slaughter houses. Our prevalence rate finding was higher than the earlier reports of 1.44% and 2.80% based also on abattoir records by [33,34] in Western-Nigeria and Ethiopia, respectively. However, a relatively similar prevalence rate of 7.95% was reported in an abattoir based-study in Western-Nigeria by [8]. Moreso, all these reports are clear indications, that the bTB is still epidemic in many African countries including Nigeria.

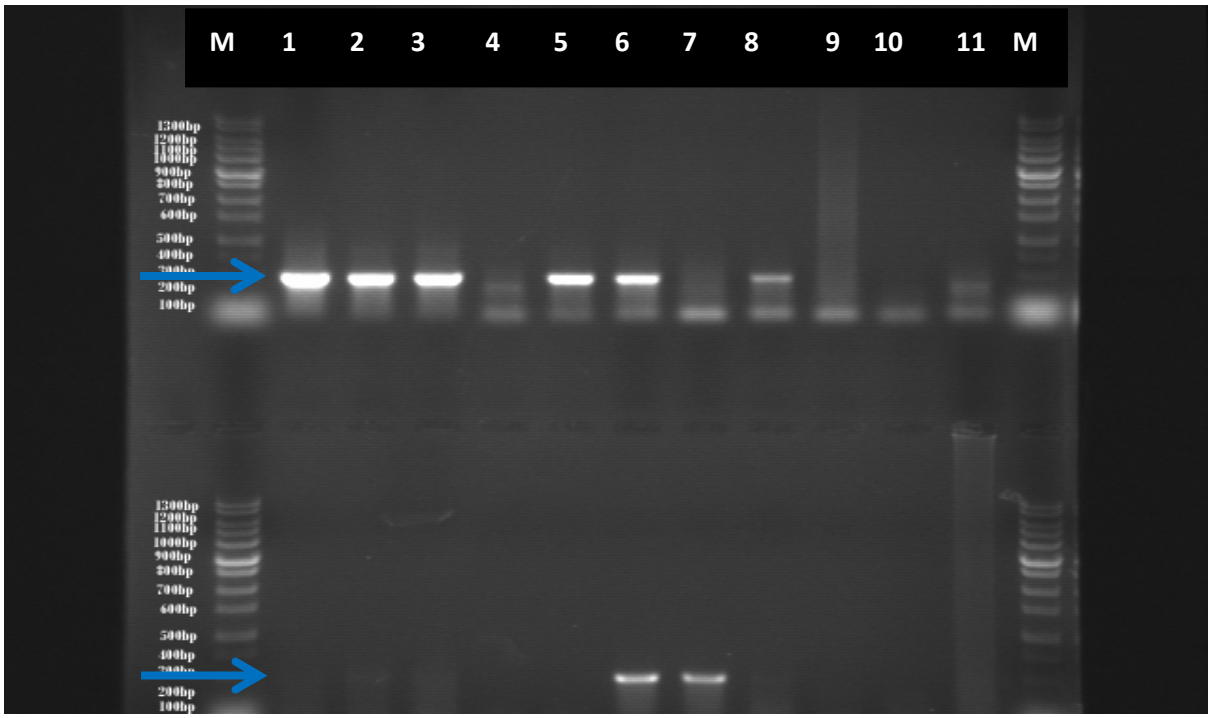

Plate 1. Agarose gel electrophoresis of *oxy*R gene amplicon

A 2-Panel agarose gel electrophoresis of PCR amplification of oxyR gene specific for M. bovis. Lane M 1.3kb Molecular weight markers (100bp DNA ladder, Bioneerlabs™, USA), ane 1 and 2 are positive controls (BCG Pasteur strain and BCG Vaccine), lane 10 negative control (nuclease free water), lane 3, 5,6,8,17 and 18 are positive samples diagnostic for M. bovis at 285bp

The present study found that suspected bTB lesions encountered in slaughtered cattle at PM may not be caused by *Mycobacterium bovis* alone. Thirty five (29.16%) of the 120 suspected bTB lesions were positive by ZN when only 10 (8.33%) were positive by PCR indicating that some of the granulomatous lesions may not be due to *M. bovis* alone as MTBC and other AFB organisms, like *Norcardia asteroides* may be involved. The prevalence rates shown in this study is much higher in female cattle than in male, 16.67% and 12.50%, respectively; however, there was no statistically significant association between sex of cattle with bTB by ZN and PCR. This was also in agreement with the expected sex-distribution pattern of the disease in cattle reported by [35]. Because female cattle stay longer in the herds than male and the fact that they are somewhat immunocompromised during pregnancy and lactation which may lead to conversion of latent TB infection to an active TB disease. A similar finding was previously reported by [32], a review of bTB in Nigeria showed there was no statistically significant association between sex of cattle with bTB by ZN.

The age-related increase in the prevalence rate shown in this study by both ZN and PCR was consistent with the chronic pattern of *tuberculosis* in cattle reported by [35,36]. Animals are increasingly becoming exposed in endemic si tuations where the infection is rampant and show increasing positivity with age [37].The statisticall y significant higher prevalence rate associatedwit h the age found in this study might have explaine d the chronic nature of the disease incattleand it i s consistent with the finding of high infection rates with increasing age of animal as reported by [38]. In addition, in stress and old age situations, a latent infection may be reactivated and lead to the development of an active disease, as reported by [35].

The agarose gel analysis of the PCR products depicted the bands of *M. bovis* targeted gene, the 285bp *oxyR gene* amplicon which is located within 16-23 rRNA regions and is known to be part of the *Ahp*C- *oxy*R regulon. A similar work was conducted in Jos, plateau State, by [39]; using DNA based multiplex PCR and ZN methods to detect MTBC in lung specimens of slaughtered cattle. In contrast to this study, they amplified 245bp fragment which is specific for MTBC complex, and 15 (30%) were positive by ZN while 9 (18%) were positive by M-PCR. Moreso, these findings were relatively higher than our infection rates recorded by both ZN and

PCR, which may be attributed to differences in the methodology (primers' specification) and sample size. However, the inability of this technique to detect the 500bp fragment in this study which has previously been reported by [40] and [39], Plateau State, Nigeria, can be attributed to the mutations which occurred along the insertion sequence of the organism, leading to the alterations of the base pair fragments, thereby preventing the specie-specific primers from amplifying the 500bp fragment; as reported by [40].

5. CONCLUSION

This study was aimed at determining the prevalence of bovine *tuberculosis* in slaughtered cattle, based on Post-Mortem (PM) meat inspection, Ziehl-Neelsen staining (ZN) and Polymerase Chain Reaction (PCR) techniques in Bauchi State, Nigeria. The present study estimated the prevalence rate of bTB in Bauchi State at 15.0%, 29.16% and 8.33%, based on PM, ZN and PCR, respectively.

The prevalence of bTB was higher (7.50%) in Bauchi metropolitan abattoir which supplies the larger population of the state with beef. Bovine TB lesions found at PM were not all due to *M. bovis* alone, as other MTBC and AFB organisms may cause bTB-like lesions which were excluded by PCR, though AFB positive by ZN.

6. RECOMMENDATION

Proper PM meat inspection should be practiced efficiently at the abattoir and slaughter houses, before taking beef to the public. The emergence MDR and XDR-TB strains are also a major concern in Nigeria. Thus, further molecular epidemiological studies with more improved techniques, like MIRU-VNTR and Spoligotyping, should be carried out on isolates from the state to look for other potential zoonotics, like *M. africanum.*

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

ACKNOWLEDGEMENTS

The authors are indebted to the Ministry of Animal Resources and Normadic Resettlement, and Area Veterinary Clinic Bauchi, Bauchi Metropolitan abattoir, Azare and Misau slaughter houses managements, and other abattoir staff Bauchi State for their cooperation and assistance during sample collection. Also to the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Radostits OM, Blood DC, Hinchey KW, Gray CC. Veterinary medicine*:* A Textbook of diseases of cattle sheep, pigs, Goats, Horses. 10th edition. Edinburgh London New York Oxford Philadelphia St Louis Sydney Toronto, Saunders Elsevier; 2007.
2. O'Reilly LM, Daborn CJ. The epidemiology of *mycobacterium* bovis infections in animals and man: A review. Tubercle Lung disease. 1995;76:1-46.
3. Shitaye JE, Tsegaye W, Pavlik I. Bovine *tuberculosis* infection in animal and human populations in Ethiopia: A review Veterinary medicine. 2007;52: 317-322.
4. WHO – World Health Organization report. Fact sheet No 104, Revised August; 2002. Available: http://www.who.int/mediacentre/factsheets/who104/en/print.html.
5. WHO- World Health Organization Report. WHO Publication on tuberculosis, Global *tuberculosis* report, 2012. Global *tuberculosis* control - surveillance, planning, financing. WHO-World Health Organization Report WHO Publication on tuberculosis, Global *tuberculosis* report, 2012. Global *tuberculosis* control - surveillance, planning, financing; 2008.
6. Shehu LM. Simple diagnosis of *tuberculosis* and tubercle bacillus in Fulani herds, Nono and some herdmen in zarira, Nigeria*.* M.sc. thesis, Ahmadu Bello University, Zaria; 1988.
7. Caffery JP. Studies of bovine *tuberculosis* eradication programme in Europe. Veterinary Microbiology. 1994;40:1-4.
8. Cadmus SIB, Adesokan HK. Causes and implications of organs/offal condemnations in some abattoirs in Western Nigeria. Tropical Animal Health Production. 2009;41:1455-1463.

9. Collins CH, Grange JM. The bovine tubercle bacillus, a review. Journal of Applied Bacteriology. 1983;55:12-29.
10. Pfeiffer U. *Tuberculosis* in Animals: clinical tuberculosis. Deviewa PD. (Ed). 3rd Edition: Armold, London; 2003.
11. Collins CH, Grange JM. Zoonotic implication of *Mycobacterium* bovis infection. International Veterinary Journal. 1987;41:363-366.
12. Thoen O. Charles Philip A, LoBue Donald A, Enarson, John B, Kaneene, andIsabel N, de Kantor. Tuberculosis: A re-emerging disease in animals and humans. Veterinar ialtaliana. 2009;45(1):135-181.
13. Kaneene JB, Pfeiffer D. Epidemiology of *Mycobacterium* bovis. In *Mycobacterium* bovis infection in animals and humans, 2nd Ed. (CO Thoen, JH Steel, MJ Gilsdorf, eds). Blackwell Publishing, Ames, lowa. 2006;44-58.
14. Gilbert N, Mitchella A, Bourn D, Mawdsley J, Clifton- Hardley R, Wint W. Cattle movement and bovine *tuberculosis* in Great Britain.Le fever, Aimy. Nature. 2005;435:491-496.
15. Srinand S, Pan X, Zhang Y, Deretic V, Musser JM. Analysis of the oxyR-ahpC Region in Isoniazid Resistant and Susceptible *Mycobacterium tuberculosis* Complex Orga nisms Recovered from Diseased Humans and Animals in Diverse Localities. Antimicrobial agents and Chemotherapy.1997;41(3):600-606.
16. Raviglione MC, Snider DE, Kochi A. Global epidemiology of tuberculosis: Morbidity and mortality of a worldwide epidemic. Journal of the American Medical Association. 1995;273:220-226.
17. Wada T, Shinji M, Atsushi H, Kazuo K. Evaluation of variable numbers of tendem repeats as molecular epidemiological markers of M. *tuberculosis* in Japan. Journal of Medical Microbiology. 2007;56:1052-1057.
18. Maher D, Van Gorkom JLC, Gondrie PCFM, Raviglione M. Community contribution to *tuberculosis* care in countries with high *tuberculosis* prevalence: Past, present and future. International Journal of *Tuberculosis* and lung diseases. 1999;3:762-768.
19. Morisky DE, Malotte CK. A Patient Education Program to Improve Rate with Anti-*tuberculosis* Drug Regimens. Health Education Quarterly.1990;17(3):253-268.
20. Bauchi state Diary. Accessed 05 September 2012. Available: http//:www.nigeriagalleria.com /nigeria/statenigeria/bauchistate. 2009;16:52.
21. FAO. Corporate Documentary Repository, Nigerian cattle population. Available: www.fao.org/docrep.Accessed 07/03/2014. 2010.
22. Census, Nigeria. Available:http://www.nigeriamasterweb.com/Nigeria06CensusFigs.html; 2006.
23. Mugo F W. Sampling in research1. Accessed 15 April; 2013. Available: http://www.socialresearchmethods.net/tutorial/mugo/tutorial.htm/08.
24. Aliyu MM, Adamu, AJ, Bilyaminu, YA. Current prevalence of tuberculous lesions among slaughtered cattle in northeastern state of Nigeria. Revue Elevage Veterinaire pays des Tropicaux. 2009;62(1):13-16.
25. Corner LA, Melville L, Kobbin K, Small, KJ, McCormy BS, Wood PR, Rothel JS. Efficacy of inspection Procedures for the detection of *tuberculosis* lesions in cattle. Australia veterinary journal. 1990;67:389–392.
26. Ron T, Ben B, Bill K, Ken C. Methods of determining Age in cattle. Cattle producer's library- CL. 2003;712:1-3.
27. Del Portillo P, Murillo LA, Patarroyo ME. Amplification of a species-specific DNA fragment of *Mycobacterium tuberculosis* and its possible use in diagnosis. Journal Clinical Microbiology. 1991;29:2163-2168.
28. Chomczynski P and Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate phenol chloroform extraction. Analytic Biochemistry. 1987;162:156–159.
29. Rosa E Romero, Daniel L Garzon, Gloria A Mejia, William Monroy, Manuel E Patarroyo, and Luis A Murillo. Identification of *Mycobacterium* bovis in Bovine Clinical Samples by PCR Species-Specific Primers. Canadian Journal of Veterinary Research.1999;63:101-106.
30. Rodriguez JG, Mejia GA, Delportillo P, Patarroyo ME, Murillo LA. Species-specific identification of *mycobacterium* bovis by PCR. Microbiology. 1995;141: 2131-2138.
31. Abubakar IA. Molecular epidemiology of human and bovine *tuberculosis* in the federal Capital Territory and Kaduna state,

Nigeria. PhD. Thesis, Plymouth University, U.K.; 2007.
32. Abubakar UB, Shehu SA, Mohammed FU. Retrospective study of *tuberculosis* in slaughtered cattle at Maiduguri abattoir, Nigeria. Medwell Journals. 2011;4(1):1-4.
33. Cadmus SIB, Alonge DO, Adesokan HK. Meat inspection and control isolate of *M. bovis* as predictors of bovine *tuberculosis* in Ibadan, Nigeria. Tropical veterinary volume. 2007;25(3):101–105.
34. Ameni G, Ameni K, Tibbo M. Bovine *tuberculosis* prevalence and risk factors assessment in cattle and cattle owners in Wuchala–Jida district, Central Ethiopia. Journal. Applied Research Veterinary Medicine. 2003;1(4):1-4.
35. Pollock JM, Neill SD. *Mycobacterium* bovis infection and *tuberculosis* in cattle. Veterinary Journal. 2002;163:115–127.
36. Kudi AC, Abubakar UB, Ndukum JA. Prevalence of bovine *tuberculosis* in camels in northern Nigeria. Journal of Camel Practice and Research. 2012;19(1):81-86.
37. Cleaveland S, Shaw DJ, Mfinanga SG, Shirima G, Kazwala RR, Eblate E, et al. M. *Mycobacterium* bovis in rural Tanzania: risk factors for infection in human and cattle populations. Tuberculosis. 2007;87(1):30-43.
38. Abubakar UB, Kudi AC, Abdulkadir IA, Okaiyeto SO, Ibrahim S. Prevalence of tuberculos is in Slaughtered Camels (*Camelus dromedarius)* Based on Post-Mortem Meat Inspection and Ziehl-Neelsen Stain in Nigeria. Journal of Camel Practice and Research. 2012;19:29-32.
39. Isioma DC, Chukwu OO, Yvonne TK, Olajide O, Chika N, Godwin OA, et al. Detection of *Mycobacterium tuberculosis* complex in Lung Specimen of Slaughtered Cattle and Goats by a DNA based multiplex Polymerase Chain Reaction and Ziehl- neelsen methods in jos, Nigeria. British Microbiology Research Journal. 2013;3(4):550-556.
40. Figueiredo EES, Junior ACC, Furlanetto LV, Silva FGS, Duarte RS, Silva JT, et al. Molecular techniques for identification of species of the *Mycobacterium tuberculosis* complex: The use of multiplex PCR and an adapted HPLC method for identification of *Mycobacterium* bovis and diagnosis of bovine tuberculosis. In: Understanding Tuberculosis-Global Experiences and Innovative Approaches to the Diagnosis. 2012;411-432.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=717&id=12&aid=6730