Molecular Characterization of Mycobacterium tuberculosis Complex in Cattle and Humans, Maiduguri, Borno State, Nigeria: a Cross-sectional Study

Ayi Vandi Kwaghe (hyelni_vandi@yahoo.com)
Federal Ministry of Agriculture and Rural Development
James Agbo Ameh
University of Abuja
Caleb Ayuba. Kudi
Ahmadu Bello University Zaria
Abdul-Ganiyu Ambali
University of Ilorin
Hezekiah Kehinde Adesokan
University of Ibadan
Victor Oluwatoyin Akinseye
University of Ibadan
Olubukola Deborah Adelakun
University of Ibadan
Joy Gararawa Usman
National Veterinary Research Institute
Simeon Idowu Cadmus
University of Ibadan

Research Article

Keywords: Cattle, Humans, Culture and isolation, Genus typing, Deletion analysis, spoligotyping, Mycobacterium tuberculosis complex

Posted Date: May 2nd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1549668/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at BMC Microbiology on January 9th, 2023. See the published version at https://doi.org/10.1186/s12866-022-02710-y.

Abstract

Introduction: In Nigeria, limited data exist to elucidate the inter-transmission of tuberculosis (TB) between humans and cattle. The study aimed at determining the circulating spoligotypes of *Mycobacterium tuberculosis* complex in cattle and humans, in Maiduguri.

Methods: We conducted a cross sectional study on bovine and human tuberculosis in Maiduguri, Borno state. We calculated sample size using the method Thrusfield and Lesions suggestive of TB from 160 slaughtered cattle was obtained from Maiduguri Central Abattoir. Sputum samples from humans; 82 abattoir workers and 147 suspected TB patients from Hospitals/clinics were obtained. Lesions and sputum samples were cultured for the isolation of *Mycobacterium* spp. Positive cultures were subjected to deletion analysis and selected isolates were spoligotyped.

Results: Fifty-two isolates were obtained from cattle. Of these, 26 (50%) belonged to *M. tuberculosis* complex (MTC) of which 17/26 (65.4 %) were characterized as *M. bovis*. In humans, seven of 12 (58.3%) MTC obtained were characterized as *M. tuberculosis*. Spoligotyping revealed SB0944 and SB1025 in cattle, while SIT838, SIT61 of LAM10_CAM and SIT1054, SIT46 of Haarlem (H) families were obtained from humans.

Conclusions: Our findings reiterate the dominance of SB0944 and SB1025 in cattle in Nigeria. We isolated *M. tuberculosis* strain of the H family mainly domiciled in Europe from humans.

Background

Bovine tuberculosis (bTB) is a chronic disease of cattle caused by *Mycobacterium bovis*. The disease has a significant impact on the international cattle trade as well as public health(1). In developing countries, laboratory diagnosis of TB is limited and often stops at the smear microscopy in humans, thus limiting the estimation of the role of *M. bovis* in human infection(2, 3). Introduction of DNA fingerprinting techniques for *M. tuberculosis* has largely enhanced the understanding of the transmission of TB(4). Differentiation of members of *M. tuberculosis* complex (MTC) is important for the accurate diagnosis of mycobacterial disease, public health surveillance and appropriate case management(5). The differentiation of MTC has become particularly important in adult and pediatric patients with human immunodeficiency virus (HIV)-related immune suppression(5) as well as occupationally exposed individuals such as abattoir workers. As reported, HIV patients infected with TB due to *M. bovis* are twice as more likely to die during treatment than those infected with *M. tuberculosis*(5).

Globally, some of the available diagnostic tools used in the speciation of MTC are deletion typing and spoligotyping. Deletion typing is a multiplex PCR technique that differentiates members of the MTC by the amplification of genomic regions of difference (RD1, RD4, RD9, and RD12) thereby identifying specific strains based on the presence and/or absence of RD-region(6). Spoligotyping is a very practical and reproducible PCR-based method, which assays the presence or the absence of a set of target sequences in the direct repeat (DR) locus(7). This technique is based on the amplification of the DR region and

subsequent differential hybridization of the amplified products with membrane-bound oligonucleotides complementary to the variable spacer regions localized between the DR's(8). Strains that are similar or different can be distinguished by their spoligotype patterns which are characterized by the number and identity of spacers(8). The presence of the spacer sequences varies in different strains and are visualized by a spot on a fixed site of the hybridization membrane(7).

There is limited data in most developing countries on the total incidence or prevalence of mycobacterial disease due to specific MTC members(9, 10) as a result of poor accessibility to molecular diagnostic techniques. In Nigeria, only few studies(11–15) has been conducted to speciate MTC despite the prevalent risk of inter-transmission between cattle and livestock workers in the country.

Maiduguri is known to domicile many of the cattle slaughtered in Nigeria as supplies of these animals are made to various parts of the country. It is also characterized by high livestock activities including slaughter and processing with concomitant human-livestock interactions. Despite the prevalence of bTB, 10.7% in cattle(16) and 0.2% in humans(17); circulating MTC strains among cattle and humans in Maiduguri are largely unknown. Our hypothesis was; the spoligotypes of MTC circulating in cattle and humans in Maiduguri is the same with the spoligotypes circulating in other parts of the country. The study aimed at characterizing MTC isolates from cattle and humans in Maiduguri, to provide important insights into the epidemiology of bTB in the area.

Materials And Methods

The aim, design and setting of the study

Maiduguri, the capital of Borno State, located in the North East region of Nigeria (Fig. 1). It is the largest city in Borno State having a population of about 1,112,449 inhabitants(18). The state shares international borders with Cameroon, Chad and Niger Republic. The major abattoir in the state is located in Maiduguri where an average of 200 cattle is slaughtered daily. The Maiduguri abattoir is the only abattoir in the metropolitan and suitable for the study because cattle from all parts of the state and across international borders (Chad republic, Niger and Cameroon) are brought to the abattoir. Data from the National Tuberculosis and Leprosy Training Programme indicates that Borno State has 252 Directly Observed Therapy Shortcourse (DOTS) Centres out of which 54 are domiciled in Maiduguri. Geographical Positioning System (GPS, GARMIN's eTrex Legend personal navigator) was used in determining the location of the sampled sites; Maiduguri Abattoir (longitude 13.17859^{O}E and latitude 11.858611^{O}N). Other study sites where sputum samples were collected include; the Chest Clinic, Sir Kashim Ibrahim Road Maiduguri (longitude 13.14565^{O}E and latitude 11.83814^{O}N); Chest Hospital Ruwan Zafi, Maiduguri (longitude 13.20222^{O}E and latitude 11.85592^{O}N); State Specialist Hospital Maiduguri (longitude 13.15013^{O}E and latitude 11.83939^{O}N); and the University of Maiduguri Teaching Hospital (UMTH) which is located along Bama road, Costin (Longitude 13.17898^{0}E and Latitude 11.82606^{O}N).

We conducted a cross sectional study of bovine and human tuberculosis. The study aimed at determining the circulating spoligotypes of *Mycobacterium tuberculosis* complex in cattle and humans, in Maiduguri. Our inclusion criteria for the study were all cattle that were taken to the abattoir for slaughter and indicated tuberculous-like lesions at postmortem while our exclusion criteria were cattle below a year that were brought to the abattoir for slaughter. For the abattoir workers, our inclusion criteria were all abattoir workers; staff, butchers, meat sellers at the abattoir that were that were willing and agreed to participate in the study. At the TB DOTS centres in the hospitals/clinics, our inclusion criteria were patients suspected to be possibly infected with TB and were requested to submit their sputum sample for analysis and consented to be part of the study. The entire period of the study was from June 2013 to September, 2015. All the research assistants in the study were trained in sample collection (lesions from tissues and organs as well as sputum), to ensure that the sample was collected in the right proportion and with limited contamination.

Sample collection, transportation and storage

The sample size used for this study was calculated according to the method described by Thrusfield(19) based on previously reported prevalence of TB in cattle(2, 20, 21), abattoir workers(13) and hospital-based study(22, 23). Lesions suggestive of bTB including lungs, liver, spleen, lymph node, kidney, heart, intestine and diaphragm in slaughtered cattle at Maiduguri Abattoir were purposively collected following detailed meat inspection over three months. During this period, sensitization campaigns were conducted among abattoir workers to encourage them to participate in the study. Following due verbal consent obtained from prospective participants, sputum samples were aseptically collected using properly labelled sterile plastic specimen containers with top screw caps. From designated hospitals and clinics for the study, three sputum samples were collected per patient (one spot sample, one-morning sample and another spot sample, which were pooled together in clean sterile well-labelled plastic containers with cock screw capss. All sputum samples were collected from the various study sites and stored at the University of Maiduguri Teaching Hospital. Finally, the samples were packaged with ice packs in Coleman transport boxes for effective transportation and transported to the Tuberculosis and Brucellosis Laboratories of the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, for processing.

Laboratory Analysis

Cattle lesions and human sputum samples were decontaminated according to earlier described procedures(24) and cultured on Lowenstein Jensen media with and without pyruvate(25). Positive cultures with acid-fast bacilli were harvested into a broth of 7H9 Middlebrook medium in microcentrifuge tubes and stored at -20^{o}C until further molecular analyses.

Mycobacterium genus typing

Heat killed mycobacterial isolates from culture-positive samples were used as DNA template and primers used are shown in Table 1. DNA amplification was done in a Thermocycler with each reaction mixture

containing 2 µl DNA template, 5 µl of Q-buffer, 10X Buffer, 25 mM $MgCl_2$, 4 µl ×10 mM dNTPs, 0.5 µl of each primer (50 pmol/ µl), 0.2 µl HotStarTaq DNA polymerase (Qiagen, Hilden, Germany) was made up to 25 µl with ultra-pure water. The reaction mixture was then heated in a Programme Thermal Controller (MyGene Series Peltier, Model MG 96^+) using the following amplification programs: 95°C for 15 min for enzyme activation, followed by 45 cycles at 94°C for 1 min for denaturation, 62°C for 1 min for annealing, and 72°C for 1 min for the extension. After the last cycle, the samples were incubated at 72°C for 10 min. Thereafter, PCR amplification products were electrophoretically separated (fractionated) in 3.0% agarose in 1Xtbe pH 8·3 at 6V/cm for 4 hours. A 1.5% agarose gel was prepared and the products were electrophoresed in 10×TAE running buffer. Ethidium bromide at a ratio of 1:5, 100 bp DNA ladder, and orange 6x loading dye were used in gel electrophoresis. Finally, bands were visualized on a UV light cabinet.

Region of Difference (RD) Deletion Typing

The PCR amplification procedures were carried out as earlier described(6). All primers used are indicated in Table 1. Briefly, each reaction mixture consisted of 1µl DNA template, 5 µl Q-buffer, 2.5 µl ×10 buffer, 2 µl 25 mM $MgCl_2$, 4 µl ×10 mM dNTPs, 0.5 µl of each primer (50 pmol/ µl), 0.125 µl HotStarTaq plus DNA polymerase (Qiagen, Hilden, Germany) and was made up to 25 µl with ultra-pure water. The reaction mixture was then heated in a Programme Thermal Controller (MyGene Series Peltier, Model MG 96^+) using the following amplification procedures: 95°C for 15 min for enzyme activation, followed by 45 cycles at 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min. After the last cycle, the samples were incubated at 72°C for 10 min. PCR amplification products were electrophoretically fractionated in 3.0% agarose in 1Xtbe pH 8·3 at 6V/cm for 4 h, and visualized by staining with ethidium bromide. Specific strains were identified based on the presence and/or absence of the genomic regions of difference (RD1, RD4, RD9, and RD12).

Table 1
Primers used for genus and deletion typing of mycobacteria isolates from Inqaba Biotech West Africa Limited, Africa's Genomic Company

Primer Name	Primer sequence	Product size
MYCGEN-F MYCGEN-R	AGAGGTTGATCCTGGCTCAG TGCACACAGGCCACAAGGGA	1030bp
MYCGEN-F MYCAV-R	AGAGGTTGATCCTGGCTCAG ACCAGAAGACATGCGTCTTG	180bp
MYCINT-F MYCGEN-R	CCTTTAGGCGCATGTCTTTA TGCACACAGGCCACAAGGGA	850bp
TB1-F TB1-R	GAACAATCCGGAGTTGACAA AGCACGCTGTCAATCATGTA	372bp
RD1	AAGCGGTTGCCGCCGACCGACC CTGGCTATATTCCTGGGCCCGG GAGGCGATCTGGCGGTTTGGGG	Present (146bp)
RD4	ATGTGCGAGCTGAGCGATG TGTACTATGCTGACCATGCG AAAGGAGCACCATCGTCCAC	Present (172bp) Absent (268bp)
RD9	CAAGTTGCCGTTTCGAGCC CAATGTTTGTTGCGCTGC GCTACCCTCGACCAAGTGTT	Present (235bp) Absent (108bp)
RD12	GGGAGCCCAGCATTTACCTC GTGTTGCGGGAATTACTCGG AGCAGGAGCGGTTGGATATTC	Present (369bp) Absent (306bp)

Spoligotyping

Spoligotyping was done at the Division of Molecular Biology and Human Genetics, Stellenbosch University, South Africa. Spoligotyping was performed on isolates identified by deletion typing as members of MTC as previously described with minor modifications(7). The direct repeat (DR) region was amplified by PCR with oligonucleotide primers derived from the DR sequence. Then, 25µl of the following reaction was used for the PCR: 12.5 µl of HotStarTag Master Mix (QIAGEN; this solution provided a final

concentration of 1.5 mM $MgCl_2$ and 200 µm each of deoxynucleoside triphosphate), 2 µl of each primer (20pmol each), 5 µl of the suspension of heat-killed cells (approximately 10 to 50ng) and 3.5 µl of distilled water. The mixture was heated for 15minutes at 96°C and subjected to 30 cycles of 1 minute at 96°C, 1 minute at 55°C and 30 seconds at 72°C. The amplified product was hybridized to a set of 43 immobilized oligonucleotides, each corresponding to one of the unique spacer DNA sequences within the DR locus. After hybridization, the membrane was washed twice for 10 minutes in 2· SSPE (1· SSPE is 0.18 M NaCl, 10 mM $NaOH_2PO4$ and 1 mM EDTA {pH 7.7})-0.5 sodium dodecyl sulfate (SDS) at 60°C and was incubated in 1:4,000-diluted streptavidin-peroxidase conjugate (Boehringer) for 45 to 60minutes at 42°C. The membrane was washed twice for 10minutes in 2 · SSPE-0.5% SDS at 42°C and rinsed with 2· SSPE for 5 minutes at room temperature. Hybridizing DNA was detected by the enhanced chemiluminescence method (Amersham) and by exposure to x-ray film (Hyper-film ECL; Amersham) as specified by the manufacturer. Patterns were numbered and prefixed with “NH” if from human isolates and “N” if isolated from cattle.

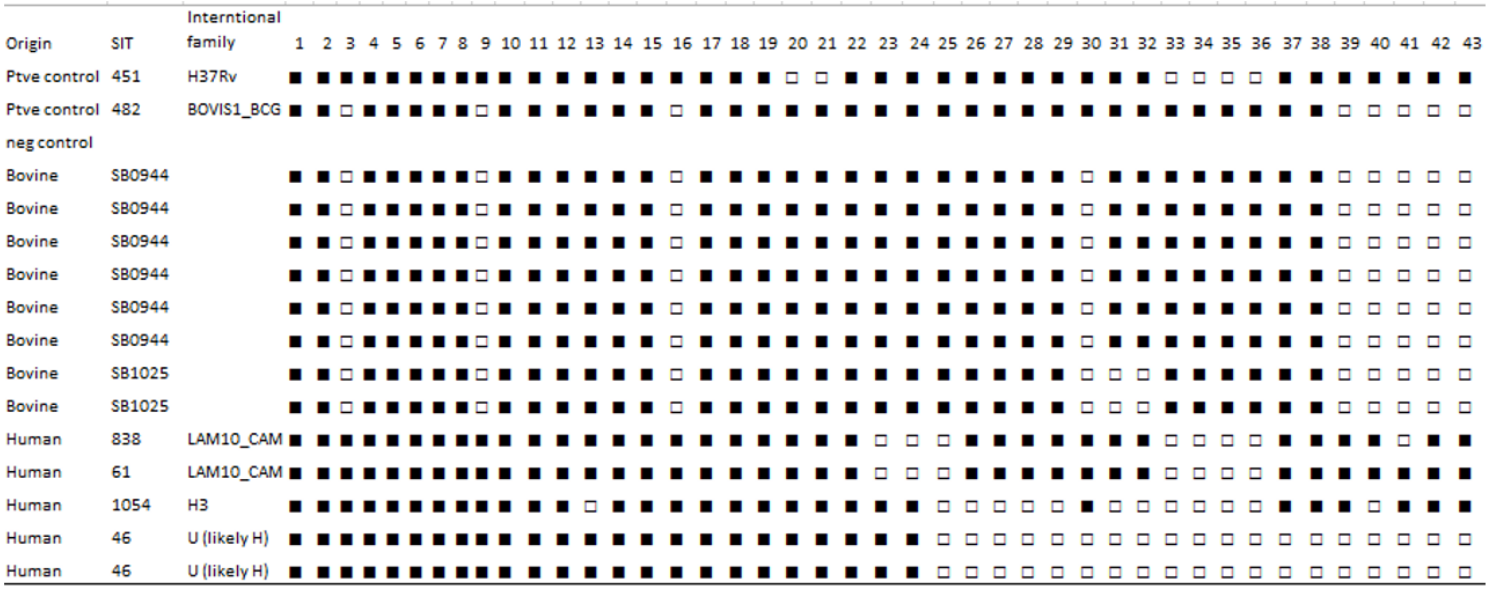
The websites, http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE/ and www.mbovis.org were used in the identification of spoligotypes. Spoligotypes were assigned to families and subfamilies by using the online tools.

Results

Out of 160 slaughtered cattle with lesions suggestive of bTB (Plate 1 & 2), 52 (32.5%) had culture-positive growths from which 26 (50%) were confirmed as belonging to MTC by genus typing (Plate 3). Further characterization by deletion typing showed that 17 of the 26 (65.4 %) isolates were *M. bovis*. In humans (229 sputum samples); 82 fand 147 from abattoir workers and patients from DOTS centres, respectively; three and nine were found to be members of MTC. Of these 12 MTC from humans, seven were characterized as *M. tuberculosis*. Spoligotyping of the selected isolates (n=12) revealed SB0944 (n=6) and SB1025 (n=2) in cattle while four spoligotypes SIT 838 and SIT 61 of LAM10_CAM as well as SIT 1054 and SIT 46 of Haarlem families were obtained from humans (Table 2).

Table 2

Spoligotypes and the international families of *M. bovis* and *M. tuberculosis* isolated from cattle and humans in Maiduguri



Discussion

The findings of this study further reiterate the endemicity of TB in both cattle and humans in Nigeria. The isolation of 65.4% *M. bovis* from slaughtered cattle in the study area corroborates earlier findings that indicated *M. bovis* as the primary agent of bTB in cattle. However, the prevalence reported in this study is lower than that of Ejeh(26) who identified 90% of the 40 isolates obtained from organs cultured as *M. bovis*. It is also lower than 99% *M. bovis* out of 180 isolates reported by other authors(12). Spoligotyping of the selected *M. bovis* isolates revealed the predominance of SB0944 in cattle in the study area. Spoligotypes SB0944 was also detected in camels a study in the same study area(27) This spoligotype pattern has been previously reported in cattle from Nigeria(15, 28), Chad, Cameroun(29), Mali(30), Morocco(31), France(32) and the United States(33). Again, the spoligotype SB1025 isolated in this study has been previously reported in Nigeria(12). As suggested(34), this spoligotype pattern could be generated from SB0944 through a single-step deletion of spacers(12) showing that spoligotypes evolve by the deletion of spacer units only. This further reiterates that SB0944 may represent the spoligotype pattern of the ancestral strain(12). Importantly, spoligotype patterns SB0944 and SB1025 belong to the African 1 (Af1) clonal complex characterized by the absence of spacer 30(35) which is also known to be widely distributed in West Africa.

Considering the zoonotic nature of *M. bovis*, spoligotype SB0944 had been previously isolated from infected sedentary and trade cattle in Ibadan(28) and from livestock traders at Akinyele Cattle Market in Ibadan(13). This indicates potential exposure of abattoir workers given prevailing factors that could enhance transmissions such as drinking of unpasteurized milk, processing infected carcasses with bare hands and unguarded close interactions with infected cattle.

Notably, the study also reported *M. tuberculosis* strains belonging to spoligotypes SIT 838 and 61 both of the international family LAM10_CAM. Earlier reports have identified LAM10_CAM in humans in Nigeria(14, 28, 36–38) and other countries in Africa including Cameroon(39), Burkina Faso(40), Sierra

Leone(41), Niger and Ivory Coast and parts of Europe(32). Previous reports showed the LAM10-CAM family as the most predominant circulating clade in Nigeria(14, 36–38, 42, 43) The LAM10-CAM was first described in Cameroon, where it represented 34% of the *M. tuberculosis* isolates in 2003(39) and has recently emerged as a dominant strain in the western province of Cameroon. Importantly, a study demonstrated LAM 10 as part of spoligotype families including LAM 1 and Beijing families which had the highest sensitivities when compared with isolates belonging to other spoligotype families; suggesting their highly clonal and homogeneous nature(44).

Again, the study reveals two isolates with spoligotypes SIT 1054 and SIT 46 belonging to the Haarlem family. Other studies also reported this spoligotype family within and outside Nigeria(14, 44, 45). The Haarlem family is considered to belong to modern strains which are known to demonstrate more virulent phenotypes compared to the ancient ones such as the East African and Indian(46). Further, reports show that the Haarlem family, of European origin, comprises nearly a quarter of the *M. tuberculosis* population in Europe, and that it also accounts for a similar proportion of strains in the Caribbean and Central America(47, 48). The Haarlem family in these regions is believed to represent a remnant of the post-Columbian European colonization(48, 49) Besides, the Haarlem strains have been associated with multidrug resistance (MDR)-TB population, indicating its ability to cause outbreaks of MDR-TB, following reports from Argentina(50), the Czech Republic(51) and Tunisia(52). The association between drug resistance (DR) and the Haarlem family has also been observed in other studies including MDR-TB cases in Tehran, Iran(53), and DR-TB cases in Hungary(54); where the rates of infection by the Haarlem genotype were 33.5% and 66.2%, respectively(54, 55).

Limitations of the study were; the abattoir workers were not randomly sampled, sampling was done purposively based on the participants' verbal consent after explaining to them the relevance of the study. Also, sputum samples collected from hospitals/clinics were based on those patients that were likely to be TB positive. This method of sampling may not be generalized, however, the sole purpose of the study was to generate the circulating spoligotypes in the area and the best population for such study is for those participants that were at high risk of being infected with the disease or those that were already indicating the clinical sign of infection. Few isolates were available for spoligotyping due to limited funds. Also, more detailed insights would have been provided if the majority of the isolates obtained were spoligotyped. Furthermore, characterization using such molecular techniques as Mycobacterial Interspersed Repetitive Units-Variable Number Tandem Repeats was not done, as this would have given better epidemiological insights into the circulating strains. It is now common knowledge that spoligotyping has limitations as a tool for the prediction of the exact phylogenetic relationships between strains of the MTC, particularly among modern strains mainly due to homoplasy(47).

Despite these limitations, however, the study reveals SB0944 and SB1025 as the circulating *M. bovis* strains in cattle and LAM 10 and Haarlem families as the circulating *M. tuberculosis* strains among humans in Maiduguri, Borno State, Nigeria.

Conclusions

In conclusion, study reveals SB0944 and SB1025 as the circulating *M. bovis* strains in cattle and LAM 10 and Haarlem families as the circulating *M. tuberculosis* strains among humans in Maiduguri, Borno State, Nigeria. Our findings further reiterate the endemicity of TB in both human and cattle populations in Maiduguri. This, therefore, calls for the need to step up control measures considering the prevailing risks for inter-transmission of TB at the human-cattle interface in the study area and Nigeria as a whole. Finally, we advocate for more extensive epidemiological studies to provide more in-depth insights into the circulating strains MTC among cattle and humans in Nigeria.

Abbreviations

Bovine tuberculosis (bTB)

Directly Observed Therapy Shortcourse (DOTS)

Human immunodeficiency virus (HIV)

Mycobacterium tuberculosis complex (MTC)

Polymerase Chain Reaction (PCR)

Tuberculosis (TB)

University of Maiduguri Teaching Hospital (UMTH)

Declarations

Ethics approval and consent to participate

Ethical clearance for the study protocol was obtained from the Research and Ethical Committee of the University of Maiduguri Teaching Hospital (UMTH). Ethical approval was subject to the National Code for Health Research Ethics (NCHRE). All methods were performed in accordance with the relevant guidelines and regulations of the NCHRE.

Written informed consent was obtained from all participants after a detailed explanation about the study was made following standard guidelines. The participants were also informed that they will remain anonymous.

Consent for publication

Participants verbal informed consent to publish was obtained by telling them our intention to publish and assured them that they will remain anonymous to the public.

Availability of data and materials

All data generated or analyzed during this study are included in the article

Competing interests

The authors declare that there is no competing interest

Funding

There was no funding for this research

Authors' contributions

AVK, JAA, CAK, AA; research concept, development of research protocol, processing of ethical clearance. SIC; contributed to the implementation of the study. AVK and JGU; sample collection. AVK, VOA, OA and HKA were involved in the processing of the samples, analysis as well as interpretation of the data. AVK, JAA and CAK; preparation of manuscript draft. All authors read, edited for intellectual content and approved the final version of the manuscript. AVK and SIC are guarantors of the paper.

Acknowledgements

We thank all participants for their cooperation during the study. We also appreciate the support provided by the management of Maiduguri Central Abattoir and the various DOTS centres in the state towards seamless conduct of the study.

References

1. Kwaghe AV, Vakuru CT, Iwar VN, Ndahi MD, Abubakar A-G, Eze E. Bovine tuberculosis: Effects and challenges faced by developed and developing countries in the eradication process. CAB Rev Perspect Agric Vet Sci Nutr Nat Resour. 2015;10.
2. Cadmus SIB, Atsanda NN, Oni SO, Akang EEU. Bovine tuberculosis in one caftle herd in Ibadan in Nigeria. Vet Med (Praha). 2004;49(11):406–12.
3. Abubakar AA, Brooks PH, Abdullahi SU, Kudi AC, Okaiyeto O. Epidemiology of bovine and human tuberculosis in the Federal Capital Territory of Nigeria, Abuja. Proc Br Soc Anim Sci. 2005;2005(September):209–209.
4. Van Soolingen D, Hermans PWM, De Haas PEW, Soll DR, Van Embden JDA. Occurrence and stability of insertion sequences in Mycobacterium tuberculosis complex strains: Evaluation of an insertion sequence-dependent DNA polymorphism as a tool in the epidemiology of tuberculosis. J Clin Microbiol. 1991;29(11):2578–86.
5. Hesseling AC, Schaaf HS, Hanekom WA, Beyers V, Cotton MF, Gie, R. P. Marais BJ, et al. Danish Bacille Calmette-Gue ´ rin Vaccine – Induced Disease in Human Immunodeficiency Virus – Infected Children. HIV/AIDS [Internet]. 2003;37(1):1226–33. Available from: https://academic.oup.com/cid/article/37/9/1226/521378

6. Warren RM, Gey Van Pittius NC, Barnard M, Hesseling A, Engelke E, De Kock M, et al. Differentiation of Mycobacterium tuberculosis complex by PCR amplification of genomic regions of difference. Int J Tuberc Lung Dis. 2006;10(7):818–22.
7. Kamerbeek J, Schouls L, Kolk A, Van Agterveld M, Van Soolingen D, Kuijper S, et al. Simultaneous detection and strain differentiation of Mycobacterium tuberculosis for diagnosis and epidemiology. J Clin Microbiol. 1997;35(4):907–14.
8. van Soolingen D, Qian L, de Haas PEW, Douglas JT, Traore H, Portaels F, et al. Predominance of a Single Genotype of Mycobacterium tuberculosis in Countries of East Asia. J Clin Microbiol. 2000;33(12):3234–8.
9. Cosivi O, Grange JM, Daborn CJ, Raviglione MC, Fujikura T. Zoonotic Tuberculosis due to Mycobacterium bovis in Developing Countries. 1999;4(1):59–70.
10. Bonard D, Msellati P, Rigouts L, Combe P, Coulibaly D, Coulibaly IM, et al. What is the meaning of repeated isolation of Mycobacterium africanum? Int J Tuberc Lung Dis. 2000;4(12):1176–80.
11. Cadmus S, Adesokan H, Adejuwon TA, Adeyemi MO. Retrospective study on bovine tuberculosis and other diseases of public health importance at Oko-Oba Abattoir, Lagos State. Trop Vet. 2010;28(1):21–30.
12. Cadmus SIB, Gordon SV, Hewinson RG, Smith NH. Exploring the use of molecular epidemiology to track bovinetuberculosis in Nigeria: An overview from 2002 to 2004. Vet Microbiol. 2011;151:133–8.
13. Adesokan HK, Jenkins AO, van Soolingen D, Cadmus SIB. Mycobacterium bovis infection in livestock workers in Ibadan, Nigeria: evidence of occupational exposure. Int J Tuberc Lung Dis. 2012;16(10):1388–1392.
14. Lawson L, Zhang J, Gomgnimbou MK, Abdurrahman ST, Le Moullec S, Mohamed F, et al. A molecular epidemiological and genetic diversity study of tuberculosis in Ibadan, Nnewi and Abuja, Nigeria. PLoS One. 2012;7(6).
15. Adesokan HK, Akinseye VO, Sulaimon MA. Knowledge and practices about zoonotic tuberculosis prevention and associated determinants amongst livestock workers in Nigeria; 2015. PLoS One. 2018;13(6):1–12.
16. FAO. The monetary impact of zoonotic diseases on society Nigeria Evidence from four zoonoses [Internet]. 2018. Available from: https://www.fao.org/3/i9001en/I9001EN.pdf
17. WHO. Global Tuberculosis Report 2017: Leave no one behind - Unite to end TB [Internet]. WHO - Technical Report Series;727. 2017. 146 p. Available from: http://www.who.int/tb/publications/global_report/gtbr2017_main_text.pdf?ua=1
18. Ibrahim UI, Mbaya AW, Geidam YA, Geidam AM. Endoparasites and Associated Worm Burden of Captive and Free-Living Ostriches (Struthio camelus) in the Semi-Arid Region of North Eastern Nigeria. Int J Poult Sci. 2006;5(12):1128–32.
19. Thrusfield M V. Veterinary Epidemiology. 2nd Editio. 108 Cowley Road, Oxford OX4 1JF, Great Britain: Blackwell Publishing; 1997. 182–183 p.

20. NASAKA J. Occurence of Bovine Tuberculosis in Slaughtered Cattle and Msc in Environment and Natural Resources Management. 2014.
21. Adedipe OD. Prevalence of Bovine tuberculosis and helminth co-infection among slaughtered cattle at Bobija municipal abattoir: Economic and public health implication. University of Ibadan, Oyo State.; 2014.
22. Zailani SB, Gabdo AH, Yusuph H, Ahidjo A, Mustapha SK, Malam SA. Prevalence of sputum smear positive tuberculosis among patients at University of Maiduguri teaching hospital. Highl Med Res J. 2005;3(2):24–30.
23. Babajide TI, Nwadike VU, Ojo DA, Onasanya OA, Ojide KC, Kula IE. Prevalence of Tuberculosis among patients attending two Secondary Hospitals in Abeokuta Ogun State. African J Clin Exp Microbiol. 2014;15(3):144–50.
24. BD Diagnostic Systems. BD BBL MycoPrep Mycobacterial Specimen Digestion/Decontamination Kit Brochure [Internet]. Vol. June. 2001. p. 2. Available from: www.bd.com/diagnostics
25. Revised National Tuberculosis Control Programme: DOTS-Plus Guidelines Jan 2010 [Internet]. Central TB Division, Directorate General of Health Services, Ministry of Health & Family Welfare, Nirman Bhavan, New Delhi – 110011; 2010. 1–122 p. Available from: papers2://publication/uuid/6B4ED336-6D98-4ED6-878D-713C5D23BAEB
26. Ejeh EF. Isolation and molecular characterization of Mycobacterium bovis in Makurdi and Otukpo Abattoirs, Benue State, Nigeria. Ahmadu Bello University Zaria, Kaduna State; 2014.
27. Lawan FA, Ejeh FE, Kwanashie C, Kadima K. Molecular characterization of *Mycobacterium bovis* isolated from camels slaughtered for human consumption in Northeastern Nigeria and the public health implication. PAMJ-OH 2020; 24 [Internet]. 2020 May 7 [cited 2022 Mar 8];2(4). Available from: https://www.one-health.panafrican-med-journal.com/content/article/2/4/full
28. Cadmus S, Palmer S, Okker M, Dale J, Gover K, Smith N, et al. Molecular Analysis of Human and Bovine Tubercle Bacilli from a Local Setting in Nigeria. J Clin Microbiol. 2006;44(1):29–34.
29. NJANPOP-LAFOURCADE, B. M. INWALD J, OSTYN A, DURAND B, HUGHES S, THOREL M, HEWINSON G, et al. Molecular Typing of Mycobacterium bovis Isolates from Cameroon. J Clin Microbiol. 2001;39(1):222–7.
30. Müller B, Steiner B, Bonfoh B, Fané A, Smith NH, Zinsstag J. Molecular characterisation of Mycobacterium bovis isolated from cattle slaughtered at the Bamako abattoir in Mali. BMC Vet Res. 2008;4:2–7.
31. Yahyaoui-Azami H, Aboukhassib H, Bouslikhane M, Berrada J, Rami S, Reinhard M, et al. Molecular characterization of bovine tuberculosis strains in two slaughterhouses in Morocco. BMC Vet Res. 2017;13(1):1–8.
32. Haddad N, Ostyn A, Karoui C, Masselot M, Thorel MF, Hughes SL, et al. Spoligotype diversity of Mycobacterium bovis strains isolated in France from 1979 to 2000. J Clin Microbiol. 2001;39(10):3623–32.

33. Driscoll JR, McGarry MA, Taber HW. DNA typing of a nonviable culture of Mycobacterium tuberculosis in a homeless shelter outbreak. J Clin Microbiol. 1999;37(1):274–5.
34. Van Embden JDA, Van Gorkom T, Kremer K, Jansen R, Van Der Zeijst BAM, Schouls LM. Genetic variation and evolutionary origin of the direct repeat locus of Mycobacterium tuberculosis complex bacteria. J Bacteriol. 2000;182(9):2393–401.
35. Müller B, Hilty M, Berg S, Garcia-Pelayo MC, Dale J, Boschiroli ML, et al. African 1, an epidemiologically important clonal complex of mycobacterium bovis dominant in Mali, Nigeria, Cameroon, and Chad. J Bacteriol. 2009;191(6):1951–60.
36. Molina-Moya B, Abdurrahman ST, Madukaji LI, Gomgnimbou MK, Spinasse L, Gomes-Fernandes M, et al. Genetic characterization of Mycobacterium tuberculosis complex isolates circulating in Abuja, Nigeria. Infect Drug Resist. 2018;11:1617–25.
37. Thumamoa BP, Asuquoa AE, Abia-Basseya LN, Lawson L, Hillc V, Zozioc T, et al. Molecular epidemiology and genetic diversity of Mycobacterium tuberculosis complex in the Cross River State, Nigeria. Infect Genet Evol. 2012;12(4):671–7.
38. Molina-moya B, Gomgnimbou MK, Spinasse L, Obasanya J, Oladimeji O, Dacombe R, et al. Mycobacterium tuberculosis complex genotypes circulating in Nigeria based on spoligotyping obtained from Ziehl-Neelsen stained slides extracted DNA. 2018;1–13.
39. Niobe-Eyangoh SN, Kuaban C, Sorlin P, Cunin P, Thonnon J, Sola C, et al. Genetic biodiversity of Mycobacterium tuberculosis complex strains from patients with pulmonary tuberculosis in Cameroon. J Clin Microbiol. 2003;41(6):2547–53.
40. Godreuil S, Torrea G, Terru D, Chevenet F, Diagbouga S, Supply P, et al. First molecular epidemiology study of Mycobacterium tuberculosis in Burkina Faso. J Clin Microbiol. 2007;45(3):921–7.
41. Homolka S, Post E, Oberhauser B, George AG, Westman L, Dafae F, et al. High genetic diversity among Mycobacterium tuberculosis complex strains from Sierra Leone. BMC Microbiol. 2008;8(February).
42. Ani A, Bruvik T, Okoh Y, Agaba P, Agbaji O, Idoko J, et al. Genetic diversity of Mycobacterium tuberculosis Complex in Jos, Nigeria. BMC Infect Dis. 2010;10.
43. Uzoewulu GN, Lawson L, Nnanna IS, Rastogi N, Goyal M. Genetic diversity of Mycobacterium tuberculosis complex strains isolated from patients with pulmonary tuberculosis in Anambra State, Nigeria. Int J Mycobacteriology. 2016;5(1):74–9.
44. Brown T, Nikolayevskyy V, Velji P, Drobniewski F. Associations between Mycobacterium tuberculosis strains and phenotypes. Emerg Infect Dis. 2010;16(2):272–80.
45. Adesokan HK, Streicher EM, van Helden PD, Warren RM, Cadmus SIB. Genetic diversity of Mycobacterium tuberculosis complex strains isolated from livestock workers and cattle in Nigeria. PLoS One. 2019;14(2).
46. Coll F, McNerney R, Guerra-Assunção JA, Glynn JR, Perdigão J, Viveiros M, et al. A robust SNP barcode for typing Mycobacterium tuberculosis complex strains. Nat Commun. 2014;5:4–8.

47. Brudey K, Gordon M, Moström P, Svensson L, Jonsson B, Sola C, et al. Molecular epidemiology of Mycobacterium tuberculosis in western Sweden. J Clin Microbiol. 2004;42(7):3046–51.
48. Filliol I, Driscoll JR, Van Soolingen D, Kreiswirth BN, Kremer K, Valétudie G, et al. Snapshot of moving and expanding clones of Mycobacterium tuberculosis and their global distribution assessed by spoligotyping in an international study. J Clin Microbiol. 2003;41(5):1963–70.
49. Sola C. Spoligotype Database of Mycobacterium tuberculosis: Biogeographic Distribution of Shared Types and Epidemiologic and Phylogenetic Perspectives. Emerg Infect Dis. 2001;7(3):390–6.
50. Ritacco V, Di Lonardo M, Reniero A, Ambroggi M, Barrera L, Dambrosi A, et al. Nosocomial spread of human immunodeficiency virus-related multidrug- resistant tuberculosis in Buenos Aires. J Infect Dis. 1997;176(3):637–42.
51. Kubín M, Havelková M, Hynčicová I, Švecová Z, Kaustová J, Kremer K, et al. A multidrug-resistant tuberculosis microepidemic caused by genetically closely related Mycobacterium tuberculosis strains. J Clin Microbiol. 1999;37(8):2715–6.
52. Mardassi H, Namouchi A, Haltiti R, Zarrouk M, Mhenni B, Karboul A, et al. Tuberculosis due to resistant Haarlem strain, Tunisia. Emerg Infect Dis. 2005;11(6):957–61.
53. Haeili M, Darban-Sarokhalil D, Fooladi AAI, Javadpour S, Hashemi A, Siavoshi F, et al. Spoligotyping and drug resistance patterns of Mycobacterium tuberculosis isolates from five provinces of Iran. Microbiologyopen. 2013;2(6):988–96.
54. Ködmön C, Niemann S, Lukács J, Sör É, Dávid S, Somosközi Á. Molecular epidemiology of drug-resistant tuberculosis in Hungary. J Clin Microbiol. 2006;44(11):4258–61.
55. Farnia P, Masjedi MR, Mirsaeidi M, Mohammadi F, Ghanavi J, Vincent V, et al. Prevalence of Haarlem I and Beijing types of Mycobacterium tuberculosis strains in Iranian and Afghan MDR-TB patients. J Infect. 2006;53(5):331–6.

Figures

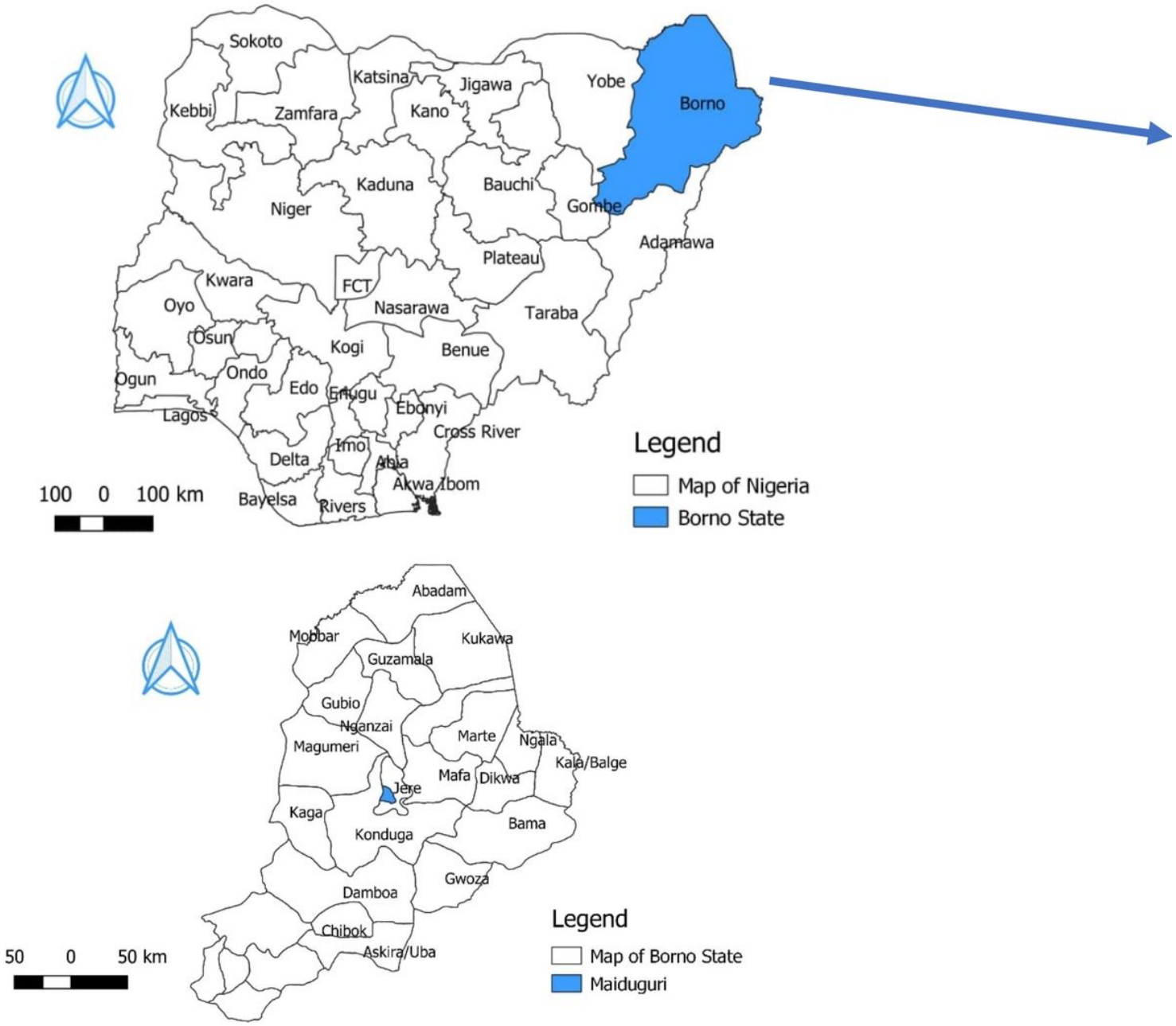


Figure 1

Map of Nigeria indicating Borno State (blue) and Map of Borno State showing Maiduguri (blue), the study area

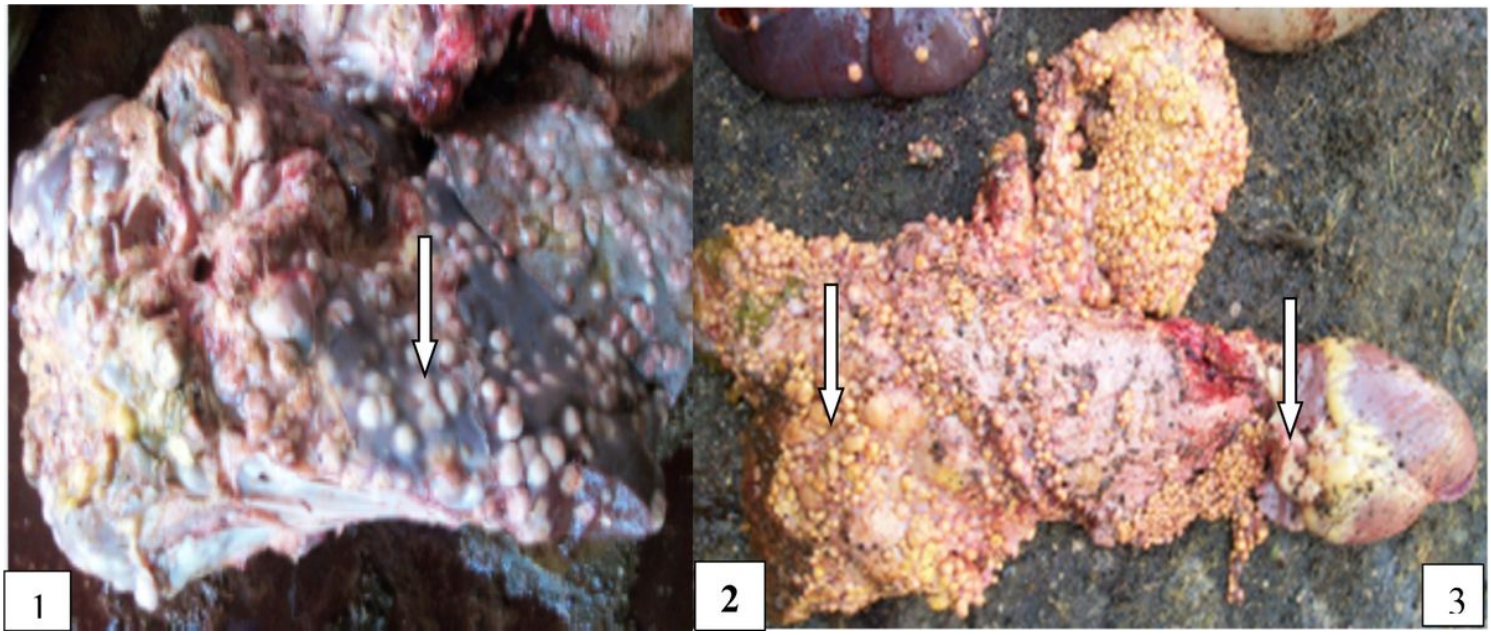


Figure 2

Plate 1: Tuberculous lesions on liver (1), lungs (2) and heart (3)

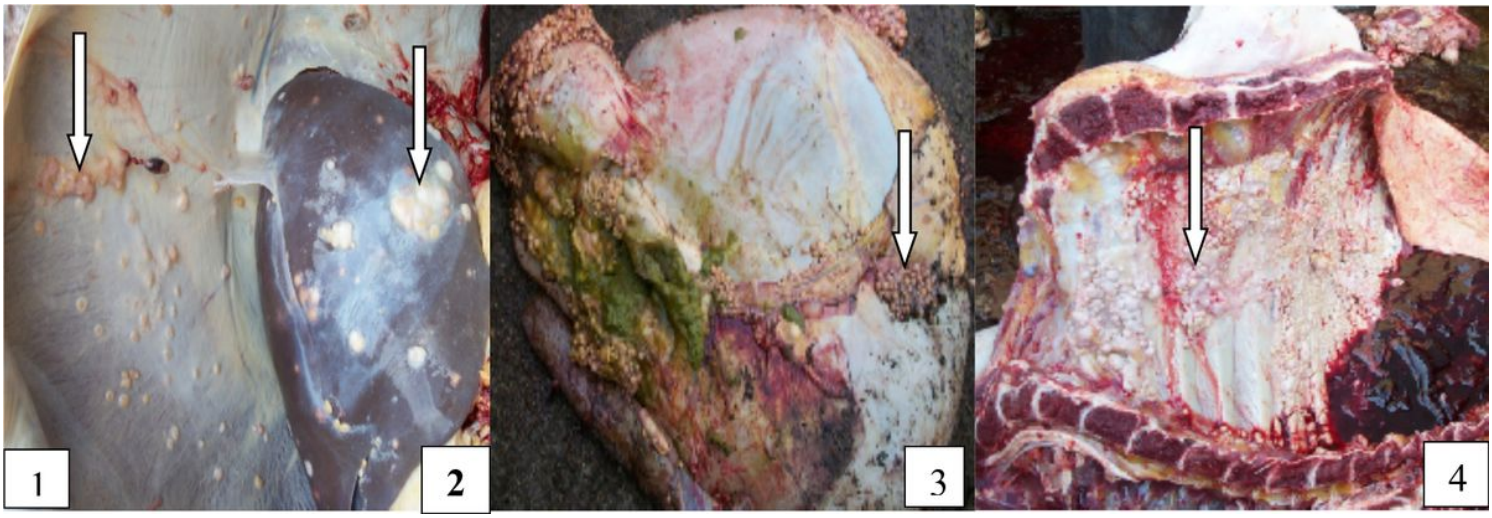


Figure 3

Plate 2 Tuberculous lesions indicated by white arrows on the diaphragm (1), liver (2), stomach (3) and chest cavity (4) observed in Maiduguri abattoir

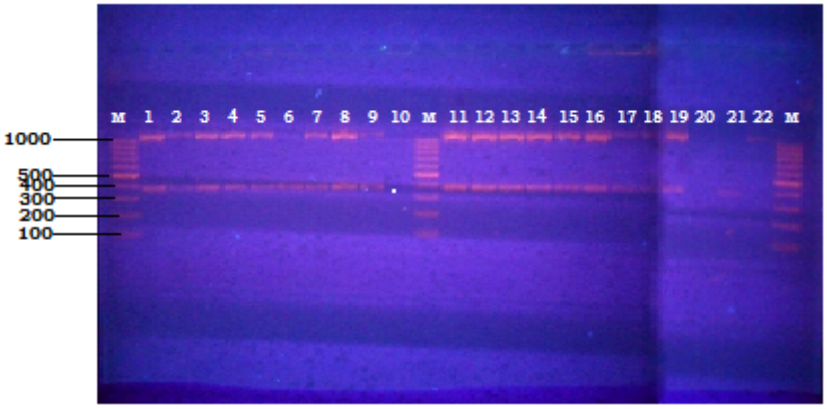


Figure 4

Plate 3: Electrophoretic fractionation of PCR products in 1.5% agar rose from the genus typing of the isolates, Lanes 1-9 and 11-19: *M. tuberculosis* complex; Lanes 10 & 20: Non-mycobacteria species, Lane 21: H37Rv: Lane M: molecular weight marker (100 bp) ladder

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- BMCSupplementary1.docx
- supplementary2.jpg
- supplemetary3.jpg