

RESEARCH

Open Access



Prevalence and molecular characterization of *Mycobacterium tuberculosis* complex in cattle and humans, Maiduguri, Borno state, Nigeria: a cross-sectional study

Ayi Vandī Kwaghe^{1,2*} , James Agbo Ameh³, Caleb Ayuba Kudi⁴, Abdul-Ganiyu Ambali⁵, Hezekiah Kehinde Adesokan⁶, Victor Oluwatoyin Akinseye^{6,7}, Olubukola Deborah Adelakun⁶, Joy Gararawa Usman⁸ and Simeon Idowu Cadmus⁶

Abstract

Introduction Globally, the highest burden of bovine and human tuberculosis resides in Africa and Asia. Tuberculosis (TB) is the second leading single infectious killer after severe acute respiratory syndrome corona virus-2 (SARSCOV-2). Bovine TB remains a treat to wild and domesticated animals, humans and hinders international trade in endemic countries like Nigeria. We aimed at determining the prevalence of bovine and human tuberculosis, and the spoligo-types 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 of Thrusfield. Lesions suggestive of TB from 160 slaughtered cattle were 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 genus typing, deletion analysis and selected isolates were spoligo-typed. Data was analysed using SPSS VERSION 16.0.

Results Prevalence of 32.5% (52/160) was obtained in cattle. Damboa local government area (LGA), where majority of the infected animals were obtained from had 35.5% bTB prevalence. All categories analysed (breed, age, sex, body conformation and score) had *P*-values that were not significant ($P > 0.05$). Sputum culture revealed a prevalence of 3.7% (3/82) from abattoir workers and 12.2% from hospitals/clinics. A significant *P*-value (0.03) was obtained when positive culture from abattoir and that of hospitals/clinics were compared. Out of the 52 culture positive isolates obtained from cattle, 26 (50%) belonged to *M. tuberculosis* complex (MTC) and 17/26 (65.4%) were characterized as *M. bovis*. In humans, 7/12 (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.

*Correspondence:

Ayi Vandī Kwaghe

hyelni_vandi@yahoo.com

Full list of author information is available at the end of the article



© The Author(s) 2022. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Conclusions Cattle in Damboa LGA need to be screened for bTB as majority of the infected animals were brought from there. Our findings revealed the presence of SB0944 and SB1025 spoligotypes from cattle in Borno state. We isolated *M. tuberculosis* strain of the H family mainly domiciled in Europe from humans.

Keywords Prevalence, Bovine and human tuberculosis, Culture and isolation, Genus typing, Deletion analysis, Spoligotyping, *Mycobacterium tuberculosis* complex

Background

Globally, in the animal sector, the highest prevalence of bovine tuberculosis (bTB) is in Africa and parts of Asia while the disease is also found in countries in Europe and the Americas [1]. The World Organisation for Animal Health through the World Animal Health Information System (WAHIS) from January 2017 to June 2018, received reports on bTB from 44% of countries associated with OIE and only a quarter of the affected countries applied relevant control measures [1]. Bovine tuberculosis is a chronic disease of cattle caused by *Mycobacterium bovis*. The disease has significant impact on the international cattle trade as well as public health [2]. *Mycobacterium bovis* can also infect and cause disease in many other mammals; humans, deer, goats, pigs, cats, dogs, and wildlife species such as wild boars, deer, and antelopes. Tuberculosis caused by *M. bovis* is not clinically distinguishable from TB due to *M. tuberculosis* [3, 4].

In humans, tuberculosis (TB) is the 13th leading cause of death and 2nd leading infectious killer after corona virus-2019 (COVID-19) [5, 6]. One quarter of the global population is infected with *Mycobacterium tuberculosis* [6]. Globally, in 2020, 10 million people were infected with TB and 1.5 million died of the disease [5] and 86% of new TB cases were from the 30 high TB burden countries with eight countries accounting for 2/3rd of the new cases; India, China, Indonesia, the Philippines, Pakistan, Nigeria, Bangladesh and South Africa [5]. There is a global decline of TB incidence with cumulative reduction rate of 11% about half of the End TB strategy of the stipulated 20% reduction from 2015 to 2020 [5]. Ending the TB epidemic by 2030 is one of the health targets of the United Nations Sustainable Development Goals (SDGs) [5].

Tuberculosis disproportionately affects people in resource-poor settings especially in Africa and Asia, posing significant challenges to developing economies as it primarily affects people during their most productive years with more than 90% of new TB cases and deaths occurring in developing countries [7]. Nigeria ranked 6th amongst the 30 high TB burden countries globally, and first in Africa [8]. Nigeria is also among the 14 countries that are in all the three WHO Global high-burden country lists for TB, TB/human immune deficiency virus (HIV) and multi-drug resistant

tuberculosis (MDR-TB) with an estimated incident rate of 219 per 100,000 population and mortality rate of 64/100,000 excluding people living with HIV. Nigeria accounts for 4% of the total TB global burden [8].

In developing countries, laboratory diagnosis of TB is often limited to the smear microscopy in humans, thus limiting the estimation of the role of *M. bovis* in human infection [9, 10]. Introduction of DNA fingerprinting techniques for *M. tuberculosis* has largely enhanced the understanding of TB transmission [11]. Differentiation of members of *M. tuberculosis* complex (MTC) is relevant for accurate diagnosis of mycobacterial diseases, public health surveillance and effective case management [12]. Differentiation of MTC has become particularly important in adult and pediatric patients with human immunodeficiency virus (HIV)-related immune suppression [12] and occupationally exposed individuals such as abattoir workers. People living with HIV and infected with TB due to *M. bovis* are twice more likely to die during treatment than those infected with *M. tuberculosis* [12].

Tuberculosis and other mycobacterial infections are major opportunistic infections in HIV/AIDS infected individuals while HIV/AIDS is a major predisposing factor for TB including reactivations of the disease [13, 14]. Even though the risk to human health is low in most developed countries, the HIV pandemic raises concern about its impact on the transmission of *M. bovis* to and between humans [15]. The highest risk groups are individuals with concomitant HIV/AIDS infection [16]. Cases of HIV-related human TB due to *M. bovis* have been reported in many developed countries [17, 18]. Limited data is available regarding the spread of bTB amongst human population in developing countries; global estimates of 2.1% of pulmonary TB and 9.4% of extra-pulmonary TB cases is attributed to *M. bovis*. Estimates of some studies in Africa attributes *M. bovis* infection to about 5–7% of all human TB cases in the region [19, 20]. Studies conducted over the past 30 years in Nigeria revealed prevalence ranges from 2.5% in 1976 to 14% in 2007 for *M. bovis*, indicating increase in the prevalence of bTB over the years [21]. Molecular analysis of mycobacterial strains isolated from both pulmonary and extra pulmonary TB cases have indicated that up to 14% of them belong to *M. bovis* [21].

Some 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 [22]. 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 [23]. 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 [24]. Strains that are similar or different can be distinguished by their spoligotype patterns which are characterized by the number and identity of spacers [24]. The presence of the spacer sequences varies in different strains and are visualized by a spot on a fixed site of the hybridization membrane [23]. In Nigeria, only few studies [25–29] have so far been conducted to classify 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 [30] and 0.2% in humans [31]; 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. We aimed at determining the prevalence of bovine and human tuberculosis and

the spoligotypes of *Mycobacterium tuberculosis* complex in cattle and humans, in Maiduguri.

Materials and methods

The 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 [32]. 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°E and latitude 11.858611°N). Other study sites where sputum samples were collected include; the Chest Clinic, Sir Kashim Ibrahim Road Maiduguri (longitude 13.14565°E and latitude 11.83814°N); Chest Hospital Ruwan Zafi, Maiduguri (longitude 13.20222°E and latitude 11.85592°N); State Specialist Hospital Maiduguri (longitude 13.15013°E and latitude 11.83939°N); and the University of Maiduguri Teaching Hospital (UMTH) which is located along Bama road, Costin (Longitude 13.17898°E and Latitude 11.82606°N).

We conducted a cross-sectional study of bovine and human tuberculosis (Fig. 2). Inclusion criteria for the study were all cattle older than a year, 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

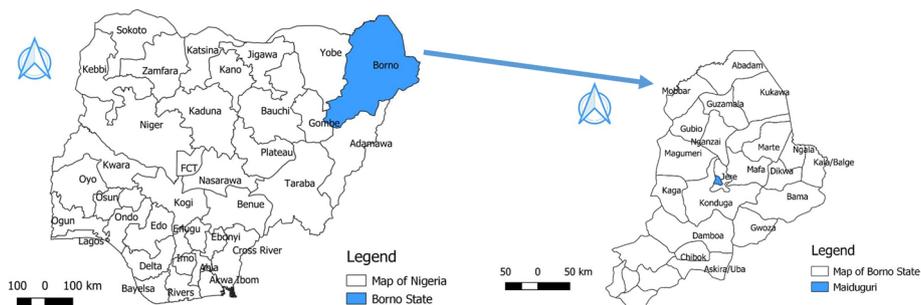


Fig. 1 Map of Nigeria indicating Borno State (blue) and Map of Borno State showing Maiduguri (blue), the study area

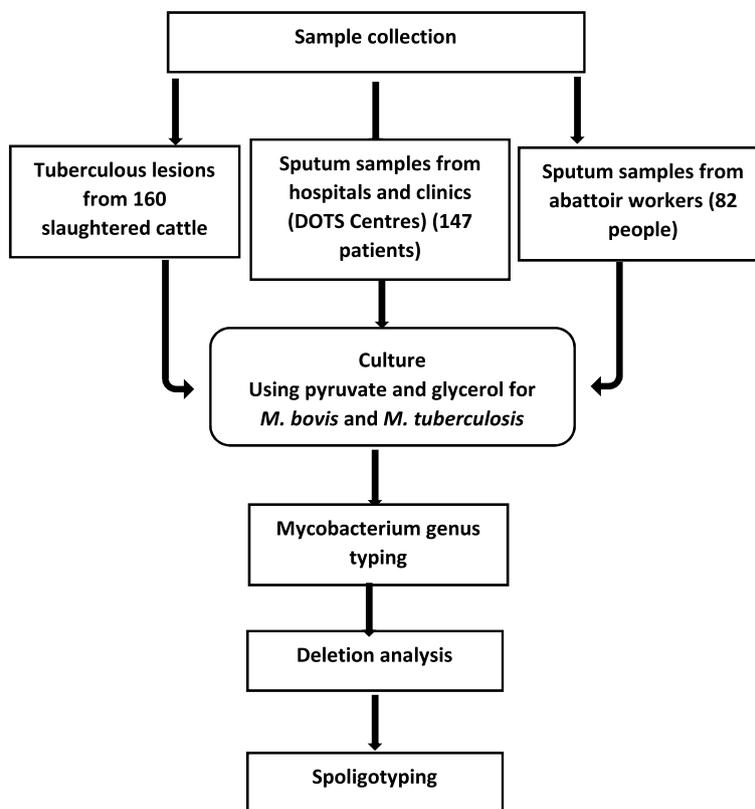


Fig. 2 Diagrammatic depiction of procedures and analysis conducted

slaughter. For the abattoir workers, our inclusion criteria were all abattoir workers; staff, butchers, meat sellers at the abattoir 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 infected with TB and were referred to the laboratory to submit their sputum sample for analysis and consented to be part of the study. Suspect case of the study were those cases that met the national TB case definitions; “a suspected pulmonary TB case is defined as any person coughing for 2 weeks or more, with or without symptoms of weight loss, tiredness, fever, night sweats, chest pain, shortness of breath, loss of appetite and coughing up blood while a suspected extra-pulmonary TB case is a person with symptoms depending on the affected organ, vertebral spine (back pain, swelling on spine); bone (long standing pain and swelling of the bone); Joints (painful joint swelling, usually affecting one joint); kidney and urinary tract (painful urination, blood in urine, frequent urination, lower back pain/loin pain); upper respiratory tract (hoarseness of voice, pain on swallowing); pleural membrane of lungs (chest pain, difficulty in breathing, fever); meninges of the brain (headache, persistent fever, neck stiffness, vomiting, irritability, convulsions, loss of consciousness); lymph node (painless swelling of the

node, may drain pus)” [33]. The entire period of the study was from June 2013 to September, 2015. Research assistants were trained on sample collection of tuberculous lesions from tissues and organs of infected cattle while those that were engaged in human sputum collection were trained on sputum sample collection, to ensure that the samples were collected properly [34].

Sample collection, transportation and storage

The sample size used for this study was calculated according to the method described by Thrusfield [$n = 1.96^2 P_{exp} (1 - P_{exp}) / d^2$; where n = required sample size, P_{exp} = expected prevalence and d = the desired absolute precision (5%)] [35] based on previously reported prevalence of TB in cattle [9, 36, 37], abattoir workers [27] and hospital-based studies [38, 39]. Samples were obtained from slaughtered cattle that had tuberculous lesions in the lungs and lymphnodes and in the case of generalized TB from other infected organs such as the, liver, spleen, kidney, heart, intestine at the Maiduguri Abattoir; lesions were obtained according to the predilection sites as observed grossly during post-mortem examination. Samples were purposively collected following detailed meat inspection over 3 months period. 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 caps. All sputum samples collected from the various study sites were 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.

Processing of samples

Cattle lesions and human sputum samples were decontaminated according to earlier described procedures [40]. Samples were processed using the BD BBL™ Mycoprep™ N-Acetyl L-Cysteine-sodium hydroxide (NALC-NaOH) decontamination method (BD BBL Mycoprep, 2000) to decontaminate and concentrate the samples to get deposits.

Preparation of the buffer and NALC reagent

The BBL™ Mycoprep™ phosphate buffer was prepared by pouring one packet of the buffer powder into a 500 ml volumetric flask. The flask was filled to the 500 ml line with sterile distilled water. The solution was transferred to a screw capped container and with the cap loosened, it was autoclaved at 121 °C for 15 minutes, cooled at room temperature and the cap tightened. The screw-cap on the Mycoprep Reagent bottle was loosened and the ample containing NALC was located, excess air from the bottle was released and the cap tightened. With the bottle held in an upright position, the bottle was squeezed until the ample broke. The bottle was shaken gently to dissolve the NALC, excessive agitation was avoided.

Decontamination test procedure

In a biological safety cabinet, equal volume of activated NALC was added to sputum sample in aerosol-free 15 ml centrifuge tube with screw cap, the centrifuge tube was capped and rocked gently until the specimen liquefied. If the sample remained viscous, more NALC reagent was added and the mixing repeated. The mixture was allowed to stand at room temperature for 15 minutes with occasional gentle shaking. Buffer of three times

(3x) equivalent of the sample was added to the mixture, mixed by rocking gently and centrifuged, for 15 minutes at 3000×g. All the supernatant were carefully decanted, small quantity of phosphate buffer was added and the sediment resuspended. The suspension was used for smear preparation and performance of mycobacteriological procedures.

The processing of lesions from tissue samples for culture was based on the OIE recommendation for digestion and decontamination procedures. The tissues were first homogenized by using a pestle and mortar as described by OIE [41], followed by decontamination in a 15 ml centrifuge tube containing equal amount of homogenized specimen and NALC (N-acetyl-L-cysteine) NaOH (containing 4% NaOH, 2.9% sodium citrate). The tube containing the mixture was allowed to stand for 15 minutes at room temperature until the specimen was digested followed by neutralization using 6 ml phosphate buffer. The mixture was then centrifuged at 3000×g for 15 minutes. The supernatant was carefully decanted; 2 ml of phosphate buffer was added to resuspend the sediment. Smears were made on the prepared culture media and incubated for culture.

Isolation of mycobacteria

Culture and isolation of *Mycobacterium* species was conducted using Lowenstein Jensen media (L-J medium), an egg-based medium, with and without pyruvate (Fig. 3) [42]. The media were prepared in duplicate – one containing 0.5% pyruvate for the isolation of *M. bovis* and the other containing glycerol for the isolation of *M. tuberculosis*. The egg medium was prepared by dissolving 37.2 g of the L-J medium base powder in 600 ml of sterile distilled water, autoclaved and allowed to cool to about 45 °C - 60 °C. Homogenised eggs based on the quantity of L-J media to be prepared were thoroughly washed in water and then cleansed with methylated spirit before breaking and was aseptically added, thoroughly mixed and distributed in 10–15 ml volumes in sterile MacCartney bottles and the caps were securely fastened. The medium was inspissated in a slanted position to coagulate the already sterile medium (Fig. 3.1).

The two media types were properly labelled, dated and inoculated in duplicate (one on pyruvate containing medium and the other on glycerol containing medium) with the final sputum/tissue sediments spread evenly on the surfaces of pairs of slopes of L-J medium using Pasteur pipettes (two drops). All inoculated slants were incubated first in a slanting position for 24 hours to allow for even distribution of inoculum at 37 °C. The bottles were then re-arranged in an upright position to increase incubator space. Bottles were arranged in chronological

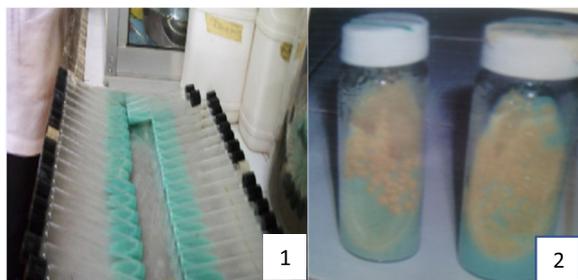


Fig. 3 Prepared L-J media before the heating of the media (1) and L-J media with growth (2)

order to make for easy examination. The bottles were incubated for 8 to 12 weeks for growth at 37°C.

Examination of mycobacterial culture slants

All cultures were examined daily for the first 7 days after incubation to detect rapidly growing mycobacteria (non tuberculous mycobacteria) and also to detect contamination. Thereafter, the cultures were examined once a week for 8 to 12 weeks to detect positive cultures of mycobacteria before adjudging the culture to be negative if there was no growth. Cultures with completely contaminated surfaces, liquified or discoloured were removed from the incubator, sterilised, discarded and excluded from the study.

Colonies from all resultant growths (Fig. 3.2) were examined for morphological appearance and acid-fast properties. Cultures with acid-fast bacilli properties were harvested into broth of 7H9 Middlebrook medium in a microcentrifuge tube and stored at -20°C until needed for further investigation. The bacterial suspension of each isolate was stained by the Z-N technique as described earlier to confirm the presence of acid-fast bacilli in the resultant growth.

Microscopy (Ziehl-Neelsen staining method)

Loopful smears of final deposits were made on clean, grease-free, duly labelled dried slides. The smear was approximately 20 mm by 10 mm, corresponding to about 100 oil immersion fields. Care was taken to ensure that the smears were not thick. The smears were left to dry naturally in the air before fixing over bunsen flame. The slides were stained by the ZN technique based on the ability of mycobacteria to retain basic dye when treated with mineral acid or an acid-alcohol solution [43]. The slides with fixed smears were arranged on staining rack over a sink. Freshly prepared carbol fuchsin was poured over the slides so that the smears were completely covered. The slides were then gently heated from below with a bunsen burner flame until steam rose and allowed to

stay for about 5 minutes. The stained slides were washed with water under running tap and excess water on the slide was drained by tilting the slides. The slides were then replaced on the rack and the decolorizer (acid-alcohol) was poured over the slide to cover the smears and allowed to act for about 3 minutes. The slides were washed under the running tap. The counter-stain (methylene-blue) was poured on the slides and left to stay for about 1 minute before washing with water under running tap. The slides were drained, arranged vertically on a slide rack and allowed to dry naturally. Dried slides were examined under the oil-immersion objective of a binocular microscope for the presence of acid fast bacilli, which appeared brick red against a blue background. The presence of AFB and cording of bacilli were indicative of *Mycobacterium* species. Positive cultures with acid-fast bacilli were harvested into a broth of 7H9 Middlebrook medium in microcentrifuge tubes and stored at -20°C until further molecular analyses.

Mycobacterial isolates from culture-positive samples were heat killed at 80°C for 1 hour and used directly as DNA template for PCR, 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 MgCl₂, 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 (Table 1). Thereafter, PCR amplification products were electrophoretically separated (fractionated) in 3.0% agarose in 1Xtbe pH8.3 at 6 V/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. The primers MYCGEN-F and MYCGEN-R generate 1030 bp for genus detection common to MTBC members were MYCAV-R and MYCGEN-F generate 180 bp for the detection of *M. avium*. MYCINT-F and MYCGEN-R generate 850 bp for detection of *M. intracellulare*. TB1-F and TB1-R generate 372 bp for *M. bovis* detection [44], Table 1.

Region of difference (RD) deletion typing

The PCR amplification procedures were carried out as earlier described [22]. All primers used are indicated in

Table 1 List of oligonucleotide sequences and cycling conditions used in the study

Primer name	Primer sequence (oligonucleotide sequences)	Product size	Enzyme activation	Cycles [45]			Incubation
				Denaturation	Annealing	Extension	
MYCGEN-F MYCGEN-R	AGAGGTTGATCCTGGCTCAG TGACACAGGCCACAAGGGA	1030 bp	95°C 15 min.	94°C 1 min	62°C 1 min	72°C 1 min	72°C 10 min.
MYCGEN-F MYCAV-R	AGAGGTTGATCCTGGCTCAG ACCAGAAGACATGCGTCTTG	180 bp	95°C 15 min.	94°C 1 min	62°C 1 min	72°C 1 min	72°C 10 min
MYCINT-F MYCGEN-R	CCTTTAGGCGCATGTCTTTA TGACACAGGCCACAAGGGA	850 bp	95°C 15 min.	94°C 1 min	62°C 1 min	72°C 1 min	72°C 10 min.
TB1-F TB1-R	GAACAATCCGGAGTTGACAA AGCACGCTGTCAATCATGTA	372 bp	95°C 15 min.	94°C 1 min	62°C 1 min	72°C 1 min	72°C 10 min.

Table 2. Each reaction mixture consisted of 1 µl DNA template, 5 µl Q-buffer, 2.5 µl × 10 buffer, 2 µl 25 mM MgCl₂, 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 (Table 2). PCR amplification products were electrophoretically fractionated in 3.0% agarose in 1Xtbe pH8-3 at 6V/cm for 4h, 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).

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 [23] (Tables 3 and 4). The direct repeat (DR) region was amplified by PCR with oligonucleotide primers derived from the DR sequence (Table 3). Then, 25 µl 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 MgCl₂ and 200 µM each of deoxynucleoside triphosphate), 2 µl of each primer (20 pmol each), 5 µl of the suspension of heat-killed cells (approximately 10 to 50 ng) and 3.5 µl of distilled water. The mixture was heated for 15 minutes 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 (Table 4), 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 NaOH₂PO₄ and 1 mM EDTA {pH7.7})-0.5 sodium dodecyl sulfate (SDS) at 60°C and was incubated in 1:4000-diluted streptavidin-peroxidase conjugate (Boehringer) for 45 to 60 minutes at 42°C. The membrane was washed twice for 10 minutes in

Table 2 List of oligonucleotide sequences and cycling conditions used in the study

Primer name	Primer sequence (oligonucleotide sequences)	Product size	enzyme activation	Cycles [45]			Incubation
				Denaturation	Annealing	Extension	
RD1	AAGCGGTTGCCCGGACCGACC CTGGCTATATTCTGGGCCCGG GAG GCGATCTGGCGGTTTGGGG	Present (146 bp)	95°C 15 min.	94°C 1 min	62°C 1 min	72°C 1 min	72°C 10 min.
RD4	ATGTGCGAGCTGAGCGATG TGTACT ATGCTGACCATGCG AAAGGAGCACCA TCGTCCAC	Present (172 bp) Absent (268 bp)	95°C 15 min.	94°C 1 min	62°C 1 min	72°C 1 min	72°C 10 min
RD9	CAAGTTGCCGTTTCGAGCC CAATGT TTGTTGCGTGC GCTACCCTCGACCAA GTGT	Present (235 bp) Absent (108 bp)	95°C 15 min.	94°C 1 min	62°C 1 min	72°C 1 min	72°C 10 min.
RD12	GGGAGCCCAGCATTACCTC GTGTTG CGGAATTACTCGG AGCAGGAGCGGT TGGATATTC	Present (369 bp) Absent (306 bp)	95°C 15 min.	94°C 1 min	62°C 1 min	72°C 1 min	72°C 10 min.

Table 3 List of oligonucleotide sequences (primers) and cycling conditions used in the study

Primer name	Primer sequence (oligonucleotide sequences)	Product size	enzyme activation	Cycles [30]			Incubation
				Denaturation	Annealing	Extension	
DRa	GGTTTTGGGTCTGACGAC, 59 biotinylated		96°C 15 min.	96°C 1 min	55°C 1 min	72°C 30 sec.	42°C 45–60 min.
DRb	CCGAGAGGGGACGAAAC		96°C 15 min.	96°C 1 min	55°C 1 min	72°C 30 sec.	42°C 45–60 min.

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.

Data analysis

Data was analyzed using STATA B and Statistical Package for the Social Sciences (SPSS) version 16.0. The 95% confidence interval was calculated online via “the confidence interval of a proportion VassarStats-vassarstats.net/prorp1.html”.

Results

One hundred and sixty slaughtered animals had tuberculous lesions; 128 (80.8%) were emaciated, 18 (11.5%) were slightly emaciated and 14 (7.7%) were looking apparently healthy (Table 5). Sixty-nine (51.9%) out of the 160 infected cattle had lesions in the lung, lymph node and one or more other organs infected (a score of 3+) as predilection site, 49 (32.7%) cattle had predilection sites of lungs and lymph node (2+) and 42 (15.4%) had predilection sites of lungs only based on gross lesions (Table 5).

One hundred and twenty-one out of 160 cattle (69.2%) between the ages of 1 to 5 had tuberculous lesions, the remaining were above 5 years of age (Table 5). Out of the 160 cattle with tuberculous lesions, 153 (96.2%) were Red Bororo (Rahaji \Abore) and other mixed breed classified as Rahaji due to their similarities, 2 each for the breeds of Bokoloji and Kuri and 3 Ambala (Arab, Bahr el or Ghazal) (Table 5). Majority of the cattle were brought from Damboa local government area (73.1%), other areas include Banki in Bama local government area (LGA) of Borno State, a town sharing border with Cameroun, Gurosaye (border

town in Cameroun), Chad Republic, Karenuwa, Mainok, Gamboru/Ngala, Damasak, Abadam, Dikwa (Mafa LGA), Dalajeri and Gudumbali (Table 5). All the categories that were analyzed based on cattle source, breed, age, sex, body conformation and score had *P*-values that were not significant (*P* > 0.05).

Out of two hundred and ninety-four (294) tissues that were cultured, 63 tissue samples (from 52 cattle) were culture positive, giving a prevalence of 32.5% (52/160) with 31.8% (49/154) prevalence in females and 50% (3/6) prevalence in males (Figs. 3, 4 and 5 & Table 5). On age ranges, 69.2% (36/52) were between the ages of 1–5 while the remaining 16 (30.8%) were > 6 years. Going by the number of culture positive cattle, 41 (78.9%) were emaciated (E), 6 (11.5%) were slightly emaciated (SE) and 5 (9.6%) were looking apparently healthy (AH). Looking at the predilection sites of infection, 8 (15.4%) out of 52 had a score of 1+ that is only the lung was affected, 18 (32.7%) had a score of 2+ (lungs and lymph node affected) and 26 (51.9%) had a score of 3+ (lungs, lymph node and any other organ affected) (Figs. 3, 4 and 5 & Table 5).

Table 6 shows that lesions from the lungs account for 35.3% of the total grossly infected organs and tissues whereas 98.6% of infected cattle had tuberculous lung lesions, followed by the lymph nodes (27.6%) while 76.9% of the cattle slaughtered had their lymph nodes infected. The list site of infection were the spleen and diaphragm (1.4%) of infected organs and 3.8% affected catted (Table 6).

A total of 147 sputum samples were analysed; 19/147(12.9%) of the suspected cases were ZN positive while (18/147)12.2% were culture positive. The prevalence of 23.8% (35/147) was obtained from both culture and ZN positive patients. Only 2 of the patients were both ZN and culture positive. For those patients that were HIV positive 6 out of 21(28.6%) of them were either ZN or culture positive. Patients with age ranges from 16 to 30 and 31–45 are worst hit by the disease having a percentage of 77.1% of those that were infected (Table 7). As the age’s progresses, the number of people infected regresses (Table 7). When the positive cases were analyzed, 22 were males (62.9%) and 13 were females (37.1%

Table 4 Sequences of the oligonucleotides used in the study

Spacer No.	Oligonucleotide sequence
1	ATAGAGGGTCGCCGGTTCGGATCA
2	CCTCATAATTGGGCGACAGCTTTTG
3	CCGTGCTTCCAGTGATCGCCTTCTA
4	ACGTCATACGCCGACCAATCATCAG
5	TTTTCTGACCACTTGTGCGGGATTA
6	CGTCGTCATTTCCGGCTTCAATTC
7	GAGGAGAGCGAGTACTCGGGGCTGC
8	CGTGAAACCGCCCCAGCCTCGCCG
9	ACTCGGAATCCCATGTGCTGACAGC
10	TCGACACCCGCTCTAGTTGACTTCC
11	GTGAGCAACGGCGGCGCAACCTGG
12	ATATCTGCTGCCCGCCGGGAGAT
13	GACCATCATTGCCATTCCTCTCCC
14	GGTGTGATGCGGATGGTCGGCTCGG
15	CTTGAATAACGCGCAGTGAATTCG
16	CGAGTTCCCCTCAGCGTCGTAATC
17	GCGCCGGCCCGCGGGATGACTCCG
18	CATGGACCCGGGCGAGCTGCAGATG
19	TAAGTGGCTTGGCGTGATCCTGGT
20	TTGACCTCGCCAGGAGAGAAGATCA
21	TCGATGTCGATGCCAATCGTCGA
22	ACCGCAGACGGCACGATTGAGACAA
23	AGCATCGCTGATGCGGTCCAGCTCG
24	CCGCCTGCTGGTGAGACGTGCTCG
25	GATCAGCGACACCACCGCACCTGTCA
26	C TTCAGCACCAACATCATCCGGCCG
27	GGATTCTGTATCTCTCCCGCGGAT
28	TGCCCCGGCGTTTAGCGATCACAA
29	AAATACAGGCTCCACGACACGACCA
30	GGTTGCCCGCGCCCTTTCCAGCC
31	TCAGACAGGTTCCGCTCGATCAAGT
32	GACCAAATAGGTATCGGCGTGTCA
33	GACATGACGGCGGTGCCGCACTTGA
34	AAGTCACCTCGCCACACCGTCGAA
35	TCCGTACGCTCGAAACGCTTCCAAC
36	CGAAATCCAGCACCATCCGCAGC
37	CGCGAATCGTCCACAGTCCCCCTT
38	CGTGGATGGCGGATGCGTGTGCGC
39	GACGATGGCCAGTAAATCGGCGTGG
40	CGCCATCTGTGCCTCATAAGGTCC
41	GGAGCTTTCCGGCTTCTATCAGGTA
42	ATGGTGGGACATGGACGAGCGCGAC
43	CGCAGAATCGCACCCGGGTGCGGGAG

Source: Kamerbeek et al. [23]

(Table 7). There was no significant *p*-value (>0.05) when results for Z-N stain and culture were compared based on sex and age groups (Tables 8 and 9). Analysis from culture positive sputum between abattoir workers and patients from hospitals/clinic indicates a significant

difference (*P* = 0.030889) in the prevalence of culture positivity between the two groups of samples (Table 10).

From the 52 (32.5%) culture-positive growths, 26 (50%) were confirmed belonging to MTC using genus typing (Fig. 6 and Table 11). Deletion typing showed that 17/26 (65.4%) isolates were *M. bovis*. In humans, 229 sputum samples were cultured, abattoir workers [46] and patients (147) from DOTS centers; three isolates from the abattoir and nine from DOTS centers were found to be members of MTC. Of these 12 MTC from humans, seven were characterized as *M. tuberculosis* (58.3%) (Table 5). Spoligotyping of the selected isolates (*n* = 12) revealed SB0944 (*n* = 6) and SB1025 (*n* = 2) in cattle (Table 12) 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 12).

Discussion

Various breeds of cattle were identified in the course of this study including Ambala which are classified as Zebu breed; Ambala is not a resident breed in the country signifying the movement and cross border trade in Borno state. Kuri breeds identified during this study are classified under Taurines as described by Blench [45]. Majority of cattle with tuberculous lesions were between the ages of 1 to 5 indicating that cattle that are in their active stage of production are more prone to bTB. Majority of cattle slaughtered in Maiduguri abattoir are the Red Bororo breed of cattle.

This study reveals higher prevalence compared to other studies; 9.6% prevalence (9.7% in males and 9.6% in females) obtained by Abubakar et al. [47] in Borno state using ZN staining method; Adedipe et al. [37], using Ziehl Neelsen technique had a prevalence of 5.5 and 8.1% when the samples were cultured from a total of 397 slaughtered cattle were examined at Bodija Municipal Abattoir located in Ibadan, Oyo State; 13.4% prevalence by Nasaka [36] in Uganda where tuberculosis like lesions were collected from cattle and cultured; 6.5% by Ekeh et al. [48] where 248 pathological lesions suggestive of bTB were collected and analyzed using Ziehl-Neelsen microscopy (ZNM); Cadmus et al. [49] had 10.5% prevalence using the simple intradermal test; and Kachalla et al. [50] obtained 17.3% prevalence where serum samples of cattle from Karu and Kubwa abattoirs in Abuja were screened for bTB.

This study has revealed the endemicity of bTB in Maiduguri. The successful culture of mycobacteria from affected tissue demonstrates the presence of generalized or miliary TB in slaughtered cattle in Maiduguri, Borno State. It further demonstrates that all lesions were not detected during routine post-mortem meat inspection at the abattoirs for bTB in carcasses which agrees with the

Table 5 Prevalence of bovine tuberculosis in relation to source, breed, age, sex, body conformation and score

Variables	Categories	N (Positive for Culture)	Total	Prevalence (%)	X ²	P Value
Sources	Abadam	1	2	50.0	14.298	0.282
	Bama	3	13	23.1		
	Chad	2	3	66.7		
	Cameroun	0	1	0.0		
	Dalagajeri	1	1	100		
	Damasak	0	2	0.0		
	Dambo	38	107	35.5		
	Dikwa	0	2	0.0		
	Gom	0	2	0.0		
	Gomboru/Ngala	1	4	25.0		
	Gubio	6	12	50.0		
	Gudumbali	0	1	0.0		
	Karenuwa	0	6	0.0		
	Mainok	0	4	0.0		
	Total		52	160		
Breed	Ambala	2	3	66.7	3.525	0.318
	Bokoloji	0	2	0.0		
	Kuri	0	2	0.0		
	Red Bororo	50	153	32.7		
Total		52	160	32.5		
Age	1–5	36	121	29.8	1.709	0.191
	5 and above	16	39	41.0		
Total		52	160	32.5		
Sex	Female	49	154	31.8	0.87	0.351
	Male	3	6	50.0		
Total		52	160	32.5		
Body Confirmation	AH	4	14	28.6	0.11	0.947
	E	42	128	32.8		
	SE	6	18	33.3		
Total		52	160	32.5		
Score	1+	8	42	19.0	4.95	0.084
	2+	17	49	34.7		
	3+	27	69	39.1		
Total		52	160	32.5		

AH Apparently Healthy, E Emaciated, SE Slightly Emaciated, Score (1⁺ = only the lungs affected, 2⁺ = lung and lymphnode infected, 3⁺ = Lung, lymphnode and any other organ infected).

P-Value ≤ 0.05 considered significant

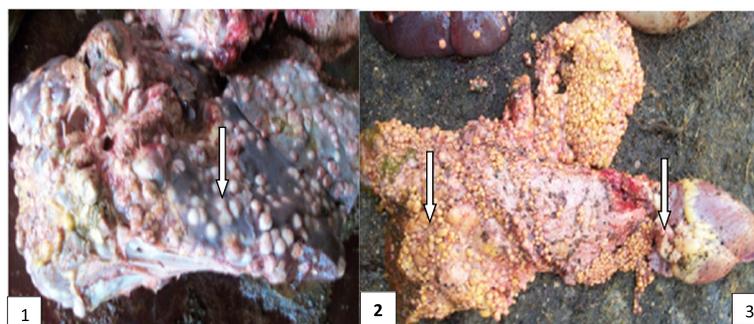


Fig. 4 Tuberculous lesions on liver (1), lungs (2) and heart (3)

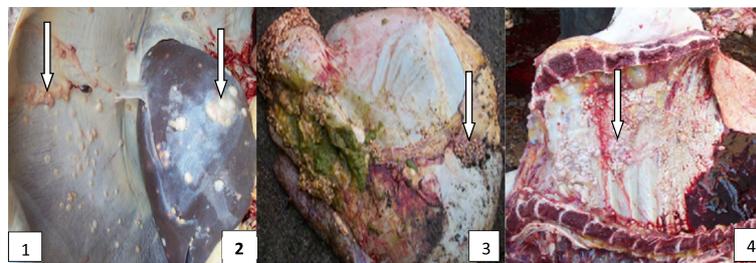


Fig. 5 Tuberculous lesions indicated by white arrows on the diaphragm (1), liver (2), stomach (3) and chest cavity (4) observed in Maiduguri abattoir

findings of Egeh [51]. The prevalence rate in males and females revealed that males are more prone to the disease than females based on the result of our culture although the values obtained were not statistically significant. This is contrary to what was obtained in terms of bTB prevalence based on the studies of Egeh et al. [48] where cows 4.8 and 1.6% for bulls; Opara et al. [52], reported cows (4.5%) than bulls, 3.3%; Cadmus et al. [9], 9.9% cows and 0.6% bulls; Egeh et al. [53], 7.4% in bulls and 9.9% in cows. Also, the study of Kachalla et al. [50], a prevalence of 29.2% in females and 13.1% in males; Adedipe

[37], revealed a prevalence of 6.7% in males and 8.3% in females.

Our finding relates that emaciation is one of the major signs in the identification of tuberculous cattle although obtained values were not statistically significant. Adult cattle with poor body conditions scores were more affected than the young adult with good body condition score. This tallies with the study of Egeh et al. [48] where a significant association was found between bTB prevalence and body conformation of the cattle that were screened. Likewise, the study of Kachalla et al. [50], also

Table 6 Indicating analysis of tissues with tuberculous lesions

S/NO	Organs/parts affected	frequency	Percentage (%) of infected organs	Percentage (%) based on the No of cattle with lesions (160)
1.	Lungs	157	35.3	98.6
2.	Heart	25	5.6	15.6
3.	Spleen	6	1.4	3.8
4.	Liver	20	4.5	12.5
5.	Kidney	9	2.0	5.6
6.	Lymph nodes	123	27.6	76.9
7.	Chest cavity	56	12.6	35.0
8.	Intestine	25	5.6	15.6
9.	Stomach	18	4.0	11.25
10.	Diaphragm	6	1.4	3.8
Total		445	100.0	

Table 7 Positive TB cases based on age category

S/NO	Age ranges	Frequency of +ve cases (Z-N + Culture)	Percentage (%)	Sex	
				Male	Female
1.	1–15	1	2.9	1	–
2.	16–30	14	40.0	7	7
3.	31–45	13	37.1	10	3
4.	46–60	5	14.3	2	3
5.	61–75	2	5.7	2	–
Total		35	100.0	22(62.9%)	13(37.1%)

+ve (positive cases), Z-N (Zielh-Nelseen)

Table 8 Ziehl-Neelsen Test results of patients from hospitals/clinic

Category	Ziehl-Neelsen		Total	X ²	P-Value
	Positive	Negative			
Sex					
F	7	63	70	1.016	0.313
M	12	65	77		
Total	19	128	147		
Age Ranges (Years)					
1–15	0	2	2	2.804	0.581
16–30	11	50	61		
31–45	5	41	46		
46–60	2	20	22		
61–75	1	15	16		
Total	19	128	147		

Table 9 Sputum culture results from hospitals/clinic

Category	Culture		Total	X ²	P-Value
	Positive	Negative			
Sex					
Females	7	63	70	0.627	0.429
Males	11	66	77		
Total	18	129	147		
Age Ranges (Years)					
1–15	1	1	2	6.88	0.142
16–30	4	57	61		
31–45	8	38	46		
46–60	4	18	22		
61–75	1	15	16		
Total	18	129	147		

indicated that body condition score is one of the measures in identifying bTB positive cattle, in the study, 50% of the seropositive cattle had poor body condition scores, 17.6% had a fair body condition score and 16.2% had good body condition score. This observation is similar to the report from Adisababa, Ethiopia by Elias et al., [54], which indicated that as body condition scores improved from poor to medium and then to good, the likelihood of positive results significantly decreased. In addition, this

result is in agreement with the findings of Ewnetu et al. [55], which reported higher bTB prevalence in cattle with medium than good body condition scores (BCS). Overall, the present result is consistent with previous reports which indicated that animals with good BCS have relatively strong immunological response to the infectious agent than animals with medium BCS and the result could also indicate the wasting nature of the disease [56]. This study also indicates that the greater proportion of the animals had a generalized form of the disease (bTB) based on identified lesions.

In Nigeria, there is inadequate meat inspection in over 90% of our abattoirs [2] and the act of trimming grossly affected parts and passing the other parts as were also observed in the Maiduguri abattoir which exposes the public and most especially those individuals that are immunocompromised such as HIV/AIDS patients to the danger of consuming infected meat. In some of the meat stands infected organs are sold to the public. Inside the abattoir building, those carcasses with generalized tuberculosis (grossly) are trimmed and washed using sponge by the butchers and taken to the stand for sale. This act also contaminates other carcasses and organs that are kept on the floor of the abattoir although Adu-bobi et al. [57] suggested that total rejection cannot be implemented fully when the abattoir management is not in the position to bear the cost of a condemned carcass.

Lungs were most affected followed by the lymph node, chest cavity, the heart and intestine. This further signifies that TB is primarily a disease of the lungs and lymph node before being disseminated to other parts of the body. Also, 12.5% of the affected cattle had lesions in their liver, 15.6% that of intestine and 76.9% of them had infected lymph nodes. This study does not tally with the studies of Opara et al. [52], where lesions were also found in 8.1% of the livers (21.5%), intestines (11.3%) and lymph nodes (11.3%) of the infected cattle.

The study revealed that patients with age ranges from 16 to 30 and 31–45 were worst hit by the disease indicating that over 70% of those that were infected fall between the age ranges of 16–45 years which is in agreement with the findings of Kwaghe et al. [58]. Also, the study of Dim

Table 10 Analysis of sputum culture results from hospitals/clinics and the abattoir

Location	Category	Culture		Total	X ²	P-Value
		Positive	Negative			
	Abattoir	3	79	82	4.6591	0.030889
	Hospital	18	129	147		
Total		21	208	229		

[69]. Again, the spoligotype SB1025 isolated in this study has been previously reported in Nigeria [26]. As suggested [70], this spoligotype pattern could be generated from SB0944 through a single-step deletion of spacers [26] 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 [26]. Importantly, spoligotype patterns SB0944 and SB1025 belong to the African I (Af1) clonal complex characterized by the absence of spacer 30 [71] 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 [64] and from livestock traders at Akinyele Cattle Market in Ibadan [27]. 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 [28, 64, 72–74] and other countries in Africa including Cameroon [75], Burkina Faso [76], Sierra Leone [77], Niger and Ivory Coast and parts of Europe [68]. Previous reports showed the LAM10-CAM family as the most predominant circulating clade in Nigeria [28, 72–74, 78, 79]. The LAM10-CAM was first described in Cameroon, where it represented 34% of the *M. tuberculosis* isolates in 2003 [75] 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 [80].

This study revealed 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 [28, 80, 81]. 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 [82]. 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 [46, 83]. The Haarlem family in these regions is believed to represent a remnant of the post-Columbian European colonization [83, 84]. 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 [85], the Czech Republic [86] and Tunisia [87]. The association between drug resistance (DR) and the Haarlem family were observed in other studies including MDR-TB cases in Tehran, Iran [88], and DR-TB cases in Hungary [89]; where the rates of infection by the Haarlem genotype were 33.5 and 66.2%, respectively [89, 90].

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 have an insight on the 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 all 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 homoplasmy [46].

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

This study reiterates that bTB IS endemic in Maiduguri with 32.5% prevalence. Majority of the infected cattle were from Damboa as demonstrated by studies of Igbokwe et al. [91]. This region of the state needs serious attention in order to control bTB in the state. Tuberculosis is also endemic in humans in the study area with a prevalence of 3.7% obtained from the abattoir workers and 12.2% from suspected TB patients from hospitals/clinic. The study concurs with majority of the studies relating TB to people in their active stage of life. The age ranges of 16–30 and 31–45 constitute the majority of those infected (77.1%). The study revealed 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. We advocate for more extensive epidemiological studies to provide more in-depth insights into the circulating strains MTC among cattle and humans in Nigeria. One Health approach should be in cooperated in dealing with the issue of human and bovine tuberculosis.

Abbreviations

bTB	Bovine tuberculosis
DOTS	Directly Observed Therapy Shortcourse
HIV	Human immunodeficiency virus
MTC	<i>Mycobacterium tuberculosis</i> complex
PCR	Polymerase Chain Reaction
TB	Tuberculosis
UMTH	University of Maiduguri Teaching Hospital

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12866-022-02710-y>.

Additional file 1: Supplementary Table 1. Spoligotype Binary, Spoligotype octal, Spoligo International Type and International family

Additional file 2: Supplementary Fig. 1. Electrophoretic fractionation of PCR products in 1.5% agarose from the genus typing of the isolates (obtained from lung lesions), Lanes 1–9 and 11–19: *M. tuberculosis* complex; Lanes 10 & 20: Negative control (Non-template control), Lane 21: H37Rv (Positive control), Lane 22: Non-tuberculous Mycobacteria (NTM), Lane M: molecular weight marker (100 bp) ladder

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.

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. The author(s) read and approved the final manuscript.

Funding

There was no funding for this research.

Availability of data and materials

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

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.

Competing interests

The authors declare that there is no competing interest.

Author details

¹Department of Veterinary and Pest Control Services, Federal Ministry of Agriculture and Rural Development, P. M. B. 135, Area 11, Garki, Abuja, Nigeria. ²Nigeria Field Epidemiology and Laboratory Training Programme, Abuja, Nigeria. ³Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Abuja, Abuja, Nigeria. ⁴Department of Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Zaria, Kaduna State, Nigeria. ⁵Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Ilorin, Ilorin, Kwara State, Nigeria. ⁶Department of Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria. ⁷Department of Chemical Sciences, Augustine University Ilara-Epe, Epe, Lagos State, Nigeria. ⁸National Veterinary Research Institute, Vom, Plateau State, Nigeria.

Received: 12 April 2022 Accepted: 21 November 2022

Published online: 09 January 2023

References

- Devi KR, Lee LJ, Yan LT, Syafinaz AN, Rosnah I, Chin VK. Occupational exposure and challenges in tackling *M. bovis* at human–animal interface: a narrative review. *Int Arch Occup Environ Health* [Internet]. 2021 Aug 1 [cited 2022 Apr 14];94(6):1147–71. Available from: <https://link.springer.com/article/10.1007/s00420-021-01677-z>
- 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(036):1–20.
- Michel AL, Huchzermeyer HFAK. The zoonotic importance of *Mycobacterium tuberculosis*: transmission from human to monkey. *J S Afr Vet Assoc*. 1998;69(2):64–5.
- Cosivi O, Grange JM, Daborn CJ, Raviglione MC, Fujikura T, Cousins D, et al. Zoonotic tuberculosis due to *Mycobacterium bovis* in developing countries. *Emerg Infect Dis*. 1998;4(1):59–70.
- World Health Organisation. Tuberculosis [Internet]. [cited 2022 May 5]. Available from: <https://www.who.int/news-room/fact-sheets/detail/tuberculosis>
- World Health Organisation. Global Tuberculosis Report, 2021. Geneva: WHO; 2021.
- Tuberculosis Highlights Nigeria Tuberculosis Fact Sheet Age and Sex Distribution. [cited 2022 Apr 22]; Available from: <http://nigeria.usembassy.gov>
- National Tuberculosis And Leprosy Control Programme. 2019 annual Tb report National Tuberculosis and. ABUJA; 2019.
- SIB C, Atsanda NN, Oni SO, EEU A. Bovine tuberculosis in one cattle herd in Ibadan in Nigeria. *Vet Med*. 2004;49(11):406–12.
- 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;2005:209.
- Van Sooling 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.
- Hesseling AC, Schaaf HS, Hanekom WA, Beyers V, Cotton MF, Gie RP, et al. Danish Bacille Calmette-Guérin vaccine – induced disease in human immunodeficiency virus – infected children. *HIV/AIDS* [Internet]. 2003;37(1):1226–33 <https://academic.oup.com/cid/article/37/9/1226/521378>.

13. Raviglione MC, Snider DE Jr, Kochi A. Global epidemicity of tuberculosis. And mortality of a worldwide epidemic. J Am Med Assoc. 1995;273(3):220–6.
14. Humblet MF, Boschiroli ML, Saegerman C. Classification of worldwide bovine tuberculosis risk factors in cattle: a stratified approach. Vet Res. 2009;40(5) <https://pubmed.ncbi.nlm.nih.gov/19497258/>.
15. Grange JM. Mycobacterium bovis infection in human beings. Tuberculosis. 2001;81(1–2):71–7.
16. Dhama K, Mahendran M, Tiwari R, Singh SD, Kumar D, Singh S, et al. Access to Research Veterinary Medicine International Volume. 2011 [cited 2019 Aug 5];14. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3135220/pdf/VM2011-712369.pdf>
17. Onder G, Rezza G, Brusaferro S. Case-fatality rate and characteristics of patients dying in relation to COVID-19 in Italy. JAMA. 2020;323:1775–6 <https://www.who.int/docs/default->
18. Report of the WHO working group on zoonotic tuberculosis (*Mycobacterium bovis*, Mainz, Germany, 14 June 1994 / with the participation of FAO [Internet]. [cited 2022 Apr 22]. Available from: <https://apps.who.int/iris/handle/10665/62534?locale-attribute=ru&mode=simple>
19. Cosivi O, Grange JM, Daborn CJ, Raviglione MC, Fujikura T. Zoonotic tuberculosis due to *Mycobacterium bovis* in developing countries. Emerging infectious diseases. 1999;4(1):59–70.
20. Anaelom NJ, Ikechukwu OJ, Sunday EW, Nnaemeka UC. Zoonotic tuberculosis : A review of epidemiology , clinical presentation , prevention and control. Journal of Public Health and Epidemiology. 2010;2(6):118–24.
21. Abubakar UB, Ameh JI, Abdulkadir IA, Salisu I, Okaiyeto SO, Kudi AC. Bovine tuberculosis in Nigeria: a review. Vet Res. 2011;4(1):24–7.
22. 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.
23. 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.
24. 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.
25. Cadmus S, Adesokan H, Adejuwon TA, Adeyemi MO. Retrospective study on bovine tuberculosis and other diseases of public health importance at Oke-Oba abattoir. Lagos State Trop Vet. 2010;28(1):21–30.
26. Cadmus SIB, Gordon SV, Hewinson RG, Smith NH. Exploring the use of molecular epidemiology to track bovine tuberculosis in Nigeria: an overview from 2002 to 2004. Vet Microbiol. 2011;151:133–8.
27. 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):1388–92.
28. 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):e38409.
29. 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.
30. 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>
31. WHO. Global tuberculosis report 2017: leave no one behind - unite to end TB. WHO - Technical Report Series. 2017;727:146 http://www.who.int/tb/publications/global_report/gtbr2017_main_text.pdf?ua=1.
32. 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 Poultry Sci. 2006;5(12):1128–32.
33. Federal Ministry of Health. National Tuberculosis and Leprosy Control Programme. (NTBLCP) National Tuberculosis, Leprosy and Buruli Ulcer Management and Control Workers Manual. 5th ed. Abuja: FMOH; 2010. <http://ntblcp.org.ng>.
34. Minnesota Department of Health tuberculosis prevention and control program. Instructions for collecting sputum for TB why is a sputum test necessary? 2021. Available from: www.health.state.mn.us/tb
35. Thrusfield MV. Veterinary Epidemiology. 2nd ed. 108 Cowley Road, Oxford OX4 1JF: Blackwell Publishing; 1997. p. 182–3.
36. Nasaka J. Occurrence of bovine tuberculosis in slaughtered cattle and risk factors to humans along the Lyantonde-Mbarara highway, Uganda. A dissertation submitted to the Directorate of Research and Graduate Training in partial fulfilment of the award of a degree of Master of Science (MSc) in Environment and Natural Resources Management. 2014. p. 1–86.
37. Adedipe OD. Prevalence of bovine tuberculosis and helminth co-infection among slaughtered cattle at Bobija municipal abattoir: economic and public health implication. Oyo State: University of Ibadan; 2014.
38. 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.
39. 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.
40. BD Diagnostic Systems. BD BBL MycoPrep Mycobacterial Specimen Digestion/Decontamination Kit Brochure. 2001. p. 2 www.bd.com/diagnostics
41. The world organisation for animal health (OIE). OIE manual of diagnostic tests and vaccines for terrestrial animals. [Internet]. OIE, Paris, France.; 2009. Available from: www.oie.int.
42. 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](https://www.doh.gov.in/publication/uuid/6B4ED336-6D98-4ED6-878D-713C5D23BAEB).
43. Koneman EW. Colour atlas and textbook of diagnostic microbiology. 4th Edition ed. Philadelphia: Lippincott Williams & Wilkins; 1992. p. 1154.
44. Deresa B, Conraths FJ, Ameni G. Abattoir-based study on the epidemiology of caprine tuberculosis in Ethiopia using conventional and molecular tools. Acta Vet Scand. 2013;55(15):7.
45. Blench R. Traditional Livestock Breeds: Geographical Distribution and Dynamics in Relation to the Ecology of West Africa. Overseas Development Institute, Portland House, Stag Place, London, SW1E 5DP. Working Paper 122. 1999. p. 1–70.
46. 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.
47. Abubakar U, Samaila D, Salisu I, Abdulkadir I, Kwaga J. Prevalence of bovine tuberculosis in slaughtered cattle based on Post-mortem meat inspection and Zeihl-Neelson stain in Borno state, Nigeria. Int J Livest Res. 2018;8(5):67.
48. Ejeh EF, Adeshokan HK, Raji MA, Bello M, Musa JA, Kudi AC, et al. Current status of bovine tuberculosis in Otukpo, Nigeria J Anim Prod Adv. 2014;4(8):501–7.
49. Cadmus SIB, Atsanda NN, Oni SO, Akang EEU. Bovine tuberculosis in one cattle herd in Ibadan in Nigeria. Vet Med (Praha). 2004;49(11):406–12.
50. Kachalla MG, Bello M, Kwaghe AV, Nguku P. The use of lateral flow technique (rapid kit test) in the determination of prevalence of bovine tuberculosis (bTB) in cattle from two abattoirs in Abuja. Nigeria Int J Life Sci. 2016;5(1):20–6.
51. Ejeh EF, Raji MA, Bello M, Lawan FA, Francis MI, Kudi AC, et al. Prevalence and direct economic losses from bovine tuberculosis in Makurdi, Nigeria. Vet. Med Int. 2014;2014:1–6.
52. Opara MN, Nwaeze CN, Olaifa AK, Maxwell JA, Okoli IC. Prevalence of bovine tuberculosis (BTB) in Imo state. Southeastern Nigeria J Top Med Parasitol. 2012;35:14–21.
53. Ejeh EF, Markus IF, Ejeh AS, Musa JA, Lawan FA, Ameh JA, et al. Seasonal prevalence of bovine tuberculosis in cattle slaughtered in Yola abattoirs. Bangladesh. J Vet Med. 2013;11(2).
54. Elias K, Hussein D, Asseged B, Wondwossen T, Gebeyehu M. Status of bovine tuberculosis in Addis Ababa dairy farms. Rev Sci Tech. 2008;27:915–23.
55. Ewnetu L, Melaku A, Birhanu A. Bovine tuberculosis prevalence in slaughtered cattle at Akaki municipal abattoir, based on meat inspection methods. Glob Vet. 2012;9(5):541–5.
56. Radostits OM, Gay CC, Kenneth WH, Constable PD. Veterinary Medicine: A textbook of the disease of cattle, horses, sheep, pigs and goats. 10th ed. Amsterdam: Elsevier Health Sciences; 2007.

57. Adu-Bobi NAK, Mak Mensah EE, Achel DG, Gyamfi OK, Bedzra KD. Preliminary investigation of bovine tuberculosis in suspected beef from a metropolitan abattoir in Ghana with Ziehl-Neelsen microscopy. *Pakistan. J Biol Sci.* 2009;12(17):1222–5.
58. Kwaghe AV, Ameh JA, Ambali AG, Gararawa JT, Bukar G, Jauro U. A retrospective studies on bovine and human tuberculosis cases in Maiduguri. *Borno state Researcher.* 2011;3(4):39–43.
59. Dim CC, Dim NR. Trends of tuberculosis prevalence and treatment outcome in an under-resourced setting: the case of Enugu state. *South East Nigeria Niger Med J.* 2013;54(6):392–7.
60. Brisibe F, Jajere SM, Isah RS. Epidemiology of pulmonary tuberculosis in Maiduguri Metropolis, northeastern Nigeria: a hospital-based retrospective study (2003–2012). *PAMJ - One Heal.* 2020;2(1). <https://doi.org/10.11604/pamj-oh.2020.2.1.22570>.
61. Kida IM, Goni BW, Ummate I, Garbati MA, Bakki B, Hammangabdo A, et al. Sputum smear positivity among patients presenting to the dots clinic. *Kanem J Med Sci.* 2017;11(2):94–8.
62. Ejeh EF. Isolation and molecular characterization of *Mycobacterium bovis* in Makurdi and Otukpo abattoirs, Benue state. *Kaduna State: Nigeria. Ahmadu Bello University Zaria;* 2014.
63. 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>
64. 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.
65. Njanpop-Lafourcade BM, Inwald J, Ostyn A, Durand B, Hughes S, Thorel M, et al. Molecular typing of *Mycobacterium bovis* isolates from Cameroon. *J Clin Microbiol.* 2001;39(1):222–7.
66. 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.
67. Yahyaoui-Azami H, Aboukhasib 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.
68. 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.
69. 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.
70. 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.
71. Müller B, Hilty M, Berg S, Garcia-Pelayo MC, Dale J, Boschirolu 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.
72. 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.
73. Thumamoa BP, Asuquo 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.
74. 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. *PLoS neglected tropical diseases.* 2018;12(2):1–13.
75. 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.
76. 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.
77. 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(1):1–8.
78. 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(1):1–5.
79. 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.
80. Brown T, Nikolayevskyy V, Velji P, Drobniwski F. Associations between *Mycobacterium tuberculosis* strains and phenotypes. *Emerg Infect Dis.* 2010;16(2):272–80.
81. 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):e0211637.
82. Coll F, McNeerney R, Guerra-Assunção JA, Glynn JR, Perdigo J, Viveiros M, et al. A robust SNP barcode for typing *Mycobacterium tuberculosis* complex strains. *Nat Commun.* 2014;5:4–8.
83. 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.
84. 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.
85. 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.
86. Kubin 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.
87. 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.
88. Haeili M, Darban-Sarokhalil D, Fooladi AAI, Javadpour S, Hashemi A, Sia-voshi F, et al. Spoligotyping and drug resistance patterns of *Mycobacterium tuberculosis* isolates from five provinces of Iran. *Microbiologyopen.* 2013;2(6):988–96.
89. Ködmön C, Niemann S, Lukács J, Sör É, Dávid S, Somoskövi Á. Molecular epidemiology of drug-resistant tuberculosis in Hungary. *J Clin Microbiol.* 2006;44(11):4258–61.
90. Farnia P, Masjedi MR, Mirsaedi 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 Inf Secur.* 2006;53(5):331–6.
91. Igbokwe IO, Madaki YI, Danburan S, Ameh JA, Aliyu MM, Nwosu CO. Prevalence of pulmonary tuberculosis lesions in cattle slaughtered in abattoirs in northeastern Nigeria. *Rev d Elev Med Vet des Pays Trop.* 2001;54(3–4):191–5.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.