

Non-tuberculous mycobacteria, not *Mycobacterium bovis*, are a significant cause of TB-like lesions observed in slaughtered cattle in Ghana

Thomas Koge Tingan^{a,b}, Gloria Ivy Mensah^{c,*}, Edward Bensa Agyekum^c, Ivy Brago Amanor^c, Samuel Ofori Addo^c, Yolanda Isabel Ayamdoo^d, Mabel Sarpong Duah^a, Lydia Mosi^{a,e}, Kennedy Kwasi Addo^c

^a West African Centre for Cell Biology of Infectious Pathogens, College of Basic and Applied Sciences, University of Ghana, Legon, Accra, Ghana

^b School of Veterinary Medicine, College of Basic and Applied Sciences, University of Ghana, Legon, Accra, Ghana

^c Department of Bacteriology, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Legon, Accra, Ghana

^d Clinical Services, Veterinary Services Directorate, Ministry of Food and Agriculture, Tamale, Ghana

^e Department of Biochemistry Cell and Molecular Biology, College of Basic and Applied Sciences, University of Ghana, Legon, Accra, Ghana

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ABSTRACT

Objectives: The aim was to isolate and identify the species of mycobacteria causing tuberculous-like (TB-like) lesions in cattle in Ghana.

Methods: Between 2019 and 2020, 68 bovine tissue samples with TB-like lesions, identified during post slaughter examination, were obtained from four major abattoirs close to border towns in Ghana. The samples were cultured on Lowenstein–Jensen medium. Isolated bacteria were characterized by Ziehl–Neelsen staining and observation for acid-fast bacilli (AFB) under a microscope. DNA was extracted from AFB-positive isolates, and mycobacterial speciation was performed by line probe assay using GenoType *Mycobacterium* CM and also with mycobacterial 16S rRNA gene amplification and sequencing.

Results: No *Mycobacterium bovis* was identified; however 53 bacterial isolates were obtained, of which 41 were non-tuberculous mycobacteria (NTM) strains and 12 were gram-positive bacteria. The predominant NTM species was *M. fortuitum* (43.9%, 18/41), with the rest being *M. novocastrense*, *M. terrae*, *M. flavescens*, *M. holsaticum*, *M. cosmeticum*, *M. virginense*, *M. intracellulare*, *M. mageritense*, *M. minnesotensis*, *M. duvalii*, *M. lehmannii*, and *M. koreense*.

Conclusions: In cattle, NTM contribute significantly to lesions observed during slaughter examination and may be an important cause of zoonotic tuberculosis. A One Health surveillance of NTM in Ghana would provide insights into their clinical significance.

1. Introduction

Bovine tuberculosis (bTB) is a chronic disease, characterized by the development of tubercles in different tissues of the infected host, particularly cattle, as well as a wide range of domestic and wild animals (Hlokwe et al., 2019; O'Reilly and Daborn, 1995; Sibhat et al., 2017). The disease is caused by species of the members of *Mycobacterium tuberculosis* complex (MTBC), primarily *Mycobacterium bovis*. There are, however, several reports about non-tuberculous mycobacteria (NTM) causing granulomatous lesions in cattle similar to the tuberculosis lesions caused by the pathogenic MTBC (King et al., 2017; Nuru et al., 2017). bTB has significant adverse effects on livestock production, while being a major cause of zoonotic tuberculosis in humans (Ayele et al., 2004;

Rodriguez-Campos et al., 2014; Tenguria et al., 2011). Developing countries, particularly in Africa and some parts of Asia, continue to suffer a severe burden of bTB with its associated zoonotic consequences for human health (Müller et al., 2013; Palmer et al., 2012). This is because unlike in developed countries, milk pasteurization is not practiced routinely, and the test and slaughter method is not strictly adhered to. This continues to contribute to the approximately 10–15% of human tuberculosis cases caused by *M. bovis* being reported in developing countries (Ashford et al., 2001; Malama et al., 2013).

In Ghana, bTB remains an epizootic disease with multiple public health and economic implications due to its zoonotic potential and the loss of revenue to cattle farmers. A national prevalence of bTB is unavailable for Ghana; however, a prevalence of 19% has been reported in cattle

* Corresponding author: Gloria Ivy Mensah, Department of Bacteriology, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Accra, Ghana.

E-mail address: gmensah@noguchi.ug.edu.gh (G.I. Mensah).

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Figure 1. The four abattoir locations (in red circles) where samples were taken.

in the North Tongu District of Volta Region (Amemor et al., 2017). Studies at slaughter facilities in Accra found a prevalence of 6.4% bTB infection among 94 cattle screened (Addo et al., 2016). In many developing countries, including Ghana, where facilities are not readily available for TB culture, the detection of acid-fast bacilli (AFB) after Ziehl–Neelsen staining of suspected lesions during necropsy examinations of cattle in various abattoirs is presumed to indicate bTB caused by *M. bovis*. Several studies have, however, reported that other mycobacterial species such as NTM are responsible for some of the tuberculous-like (TB-like) lesions in cattle (Katale et al., 2014; Kuria et al., 2018; Nuru et al., 2017). In Ghana, no study has fully investigated the aetiology of TB-like lesions observed in cattle during post slaughter examination and their zoonotic potential. This information is of critical and timely importance, because some studies have reported NTM infections in human tuberculosis cases (Addo et al., 2017; Otchere et al., 2017). The aim of this study was therefore to isolate and characterize mycobacterial species from TB-like lesions of slaughtered cattle in Ghana.

2. Methods

2.1. Study site

Samples were collected from four government-certified abattoirs: Tema (GIHOC) and JFAMCO abattoirs in the Greater Accra Region, Tamale Abattoir in the Northern Region, and Ho abattoir in the Volta Region (Figure 1). Tamale and Ho abattoirs were selected because of their proximity to borders of neighbouring countries – Burkina Faso, Mali, Niger, and Togo, from where most cattle are imported into Ghana. The daily average slaughter for the abattoirs are as follows: Tamale $n = 60$, Ho $n = 10$, Madina/Accra $n = 20$, and Tema $n = 25$. Cattle slaughtered at these abattoirs are brought in by butchers who purchase them from various locations within the country or import them from neighbouring countries.

2.2. Breed of cattle

In Ghana, the West African Shorthorn or WASH (*Bos taurus brachyceros*) remains the major breed of cattle and is estimated to be about 60% of the cattle population. Thus, this breed is the most traded in domestic stocks (Atiadeve et al., 2014). Other breeds such as Sanga (*Bos taurus*

africanus) and Zebu (*Bos primigenius indicus*) are also significant in the trade, but these are mostly imported or cross-breeds.

2.3. Data collection

The age, sex, type (class), and breed of cattle were recorded. Cattle were classified as young (<6 years) or old (>6 years) based on dentition characteristics (Pace and Wakeman, 2003) and/or farm records. The geographic origin of the animals and the system under which they were managed were also recorded. The organs or tissues from which the lesions originated were recorded.

2.4. Sample collection

As standard practice at the abattoirs, certified veterinary officers perform ante-mortem examinations on all animals before slaughter. Post-mortem inspections are then conducted after slaughter, where the carcasses are examined for suspected TB-like lesions that are indicative of tuberculosis infection.

From December 2019 to March 2020, 68 bovine tissue samples were collected from 50 individual cattle after detailed necropsy examinations for the detection of gross macroscopic lesions compatible with bTB (Figure 2) using procedures described previously (Demelash et al., 2009). The samples were stored at -20°C until processing for culture of mycobacteria at the pathogen level 3 laboratory of Noguchi Memorial Institute for Medical Research.

2.5. Tissue processing and culture

Prior to culture procedures, direct smears from lesions of each tissue sample were made and stained with Ziehl–Neelsen stain for the detection of AFB. Each tissue sample with lesions was chopped into smaller pieces in a sterile large glass petri dish and 10 grams of the pieces were then weighed into a Stomacher bag. Twenty millilitres of phosphate buffered saline (PBS) was added and the tissue sample macerated for 10 minutes using a laboratory blender (Stomacher). Ten millilitres of the homogenized mixture of tissue sample and PBS was filtered through cheesecloth into a 50-ml Falcon tube, and 10 ml of 0.5% *N*-acetyl-L-cysteine/2% NaOH/1.45% Na-citrate solution (decontaminating solution) was added. The mixture was then left at room temperature for 20 minutes after which it was topped up with PBS to the 50 ml mark and centrifuged at 3500 rpm for 15 minutes.

The supernatant was decanted while the pellet was resuspended in 2 ml PBS for inoculation onto culture media. A volume of 200 μl of the suspension was plated on two Lowenstein–Jensen slants, one containing pyruvate and the other containing glycerol, and incubated at 37°C . Cultures were observed daily for the first week to identify fast growers and then weekly for visible growth of bacteria until 12 weeks. Smears were prepared for each viable growth and Ziehl–Neelsen staining was performed to confirm the presence of AFB.

2.6. DNA extraction

A loopful of AFB in pure culture growing at the log phase was suspended in 1 ml of sterile distilled water and heated at 95°C for 1 hour to allow for the disruption of the mycobacterial cell wall to release DNA into suspension. The resulting suspension was stored at -20°C and used for all downstream DNA-based assays.

2.7. Speciation of mycobacteria using a line probe assay (LPA)

The mycobacterial species characterization was initially done on the extracted DNA samples using LPAs from Hain Lifescience GmbH, Germany: GenoType MTBC (for speciation of members of the MTBC) and GenoType *Mycobacterium* CM (for speciation of common NTM). The assays were performed using the reagents provided and in accordance with

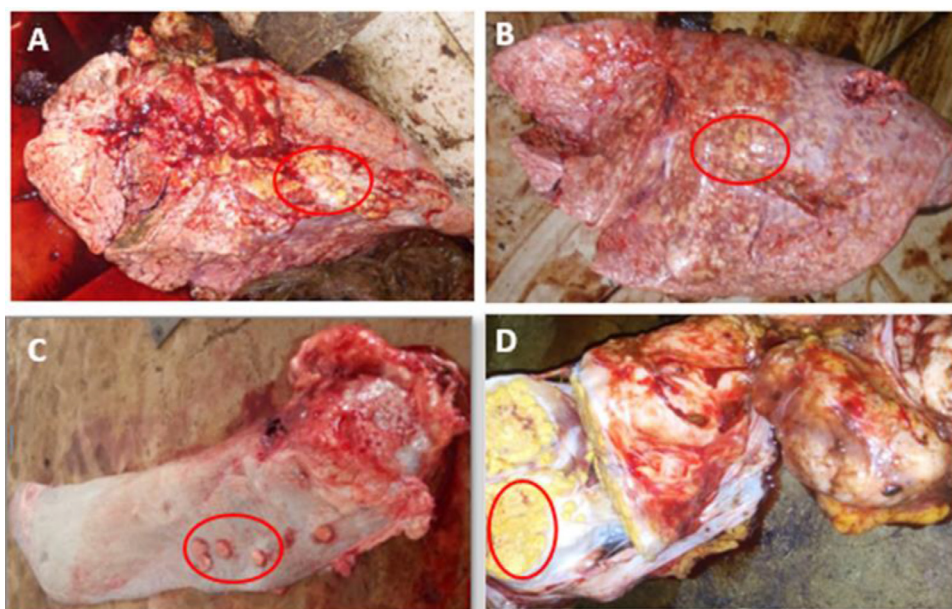


Figure 2. Macroscopic lesions (shown in red circles) of cattle tissue compatible with bovine tuberculosis: (A) (B) lungs, (C) spleen, and (D) lymph node.

the manufacturer's instructions. Each assay is made up of two steps: an amplification step, which is a multiplex PCR, followed by a reverse hybridization.

A positive control sample containing the Control DNA (C+) provided, which is *M. kansasii* DNA, was used as the positive control, and sterile nuclease-free water was used as the negative control in place of the templates. Hybridization was performed on a TwinCubator and the resulting banding patterns were compared to the reference chart provided by the manufacturer. The species of the mycobacteria were then determined based on the interpretation or reference charts of *Mycobacterium* genotype MTBC and CM, as provided by the manufacturer.

2.8. Amplification of the *Mycobacterium* 16S rRNA gene

To identify the isolates that could not be identified by the LPAs and to confirm those that were identified, the 16S mycobacterial rRNA gene was amplified in PCR reactions using specific primers: 5'-AGAGTTTGATCCTGGCTCAG-3' and 5'-AAAAAGCGACAAACCCTACGAG-3'. The PCR mix contained 18 µl nuclease-free water (H₂O), 25 µl of OneTaq Quick-Load DNA polymerase with buffer (2 µM), 1 µl forward primer PA (10 µM), 1 µl reverse primer MSHA (10 µM), and 5 µl of template, making 50 µl PCR mix. A negative control (no template) and a positive control (DNA from *M. tuberculosis* H37Rv) were included to assay for contamination of the reagents and successful PCR amplification, respectively. Amplification was done by preheating at 95°C, initial denaturation at 94°C for 5 minutes, 45 seconds of secondary denaturation at 94°C, followed by 35 cycles of annealing for 45 seconds at 56°C, followed by 45 seconds of elongation at 72°C, and then a final elongation for 10 minutes at 72°C. The amplicons were resolved on a 2% gel, and a band size of 550 bp was observed.

2.9. Sequencing of mycobacterial 16S rRNA gene

The positive amplicons were then outsourced for Sanger sequencing of the mycobacterial 16S rRNA gene. The nucleotide sequences obtained for each amplicon were cleaned using MEGA version 7 (open-source) software and the Staden package, to obtain a chromatogram of nucleotides with sharp peaks that were utilized in the Blast search for the identification of the organism with highest identity score on

the National Centre for Biotechnology Information (NCBI) website (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

2.10. Phylogenetic analysis of mycobacterial 16S rRNA gene sequences

The trace files from the Sanger sequencing were processed using SeqTrace 0.9.0 (Stucky, 2012). The files were aligned and consensus sequences were computed from matching forward and reverse traces, low-quality base calls were filtered out, and ends of sequences were trimmed. Consensus DNA sequences were then exported to FASTA files. These sequences were aligned with Clustal Omega (Sievers et al., 2011) and cleaned with GBlocks (Talavera and Castresana, 2007) using the default parameters to remove non-informative and gapped sites. The cleaned non-zero length alignments were then concatenated and exported in NEXUS file format. These files were imported into raxmlGUI (Edler et al., 2020) for phylogenetic tree construction with maximum likelihood algorithm using thorough bootstrapping with 1000 replicates. The tree was aesthetically customized thereafter with FigTree v1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>).

3. Results

3.1. Characteristics of the cattle sampled for the study

The majority of the 50 sampled cattle were female (60%, 30/50) and of the WASH breed ($n = 29$), followed by Sanga ($n = 10$), Gudali ($n = 3$), N'dama ($n = 3$), Muturu ($n = 2$), White Fulani ($n = 2$), and Zebu ($n = 1$). The organ with the most TB-like lesions was the lung (57.3%, 39/68), followed by the liver ($n = 12$), lymph nodes ($n = 7$), spleen ($n = 4$), intestines ($n = 5$), and udder ($n = 1$). Direct smears from lesions of the 68 tissue samples yielded 48.5% (33/68) positivity for AFB, while positivity was 69.1% (47/68) after culture. Overall, 53 individual bacterial isolates were obtained from the 68 tissues samples after culture and were used for further studies.

3.2. *Mycobacterium* species identified using GenoType MTBC and GenoType *Mycobacterium* CM

The DNA of the 53 culture-positive samples was subjected to LPA, and while no MTBC species was identified, 17 of the isolates were identified as *M. fortuitum* and one as *M. szulgai* by the GenoType *Mycobacterium*

Table 1
Mycobacterium species identified using GenoType Mycobacterium CM

Sample ID	Species identified
JA004 Lungs	<i>M. fortuitum</i>
NA001 Lungs	<i>M. fortuitum</i>
NA002 Lungs	<i>M. fortuitum</i>
NA002 Spleen	<i>M. fortuitum</i>
NA005 Lungs	<i>M. fortuitum</i>
NA010 Lungs	<i>M. fortuitum</i>
NA011 Lungs	<i>M. szulgai</i>
NA013 Lungs	<i>M. fortuitum</i>
NA016 Lungs	<i>M. fortuitum</i>
NA019 Lungs	<i>M. fortuitum</i>
NA020 Udder	<i>M. fortuitum</i>
NA021 Lungs	<i>M. fortuitum</i>
NA028 Lungs	<i>M. fortuitum</i>
NA029 Liver	<i>M. fortuitum</i>
NA030 Liver	<i>M. fortuitum</i>
NA030 Liver	<i>M. fortuitum</i>
TA002 Liver	<i>M. fortuitum</i>
HA007 Lymph Nodes	<i>M. fortuitum</i>

Table 2
Gram-positive bacterial species identified from 16S rRNA sequencing

Gram-positive bacterial species identified	Frequency	Percentage (%)
<i>Nocardia flavorosea</i>	2	3.77
<i>Nocardia farcinica</i>	2	3.77
<i>Cellulosimicrobium cellulans</i>	4	7.54
<i>Gordonia bronchialis</i>	2	3.77
<i>Dietzia cinnamea</i>	1	1.89
<i>Kocuria atrinae</i>	1	1.89
Total	12/53	22.64

CM (Table 1). However, the remaining isolates ($n = 35$), the majority of which were presumed to be mycobacterial strains and a few gram-positive bacteria (GPB), could not be identified using the LPA method.

3.3. Mycobacterial species identified using 16S rRNA sequencing

Since most of the isolates could not be identified by the LPA method (GenoType MTBC and GenoType Mycobacterium CM), all 53 isolates were further analysed by sequencing the mycobacterial 16S rRNA gene, after amplification. Blast searches of the gene sequences on the NCBI website revealed no identification of MTBC species among the isolates. Among the 53 bacterial isolates, 41 were identified as NTM and the others ($n = 12$) were identified as GPB (Table 2). The 41 NTM isolates belonged to one of 13 different species of mycobacteria, with the predominant species being *M. fortuitum* (43.9%, 18/41). The others were *M. novocastrense*, *M. terrae*, *M. flavescens*, *M. holsaticum*, *M. cosmeticum*, *M. virginense*, *M. intracellulare*, *M. mageritense*, *M. minnesotensis*, *M. duvalii*, *M. lehmannii*, and *M. koreense* (Table 3).

3.4. Concordance between LPA and 16S rRNA sequencing for speciation of mycobacteria

Comparing the 18 isolates of mycobacteria identified by LPA to the same isolates identified by 16S rRNA sequencing, 10 out of 18 isolates were identified by both GenoType Mycobacterium CM and 16S rRNA sequencing as *M. fortuitum* (10/18, 55.6% concordance) (Table 4). The GenoType Mycobacterium CM results were discordant with the 16S rRNA sequencing results for the remaining eight isolates. Four of the isolates were identified by 16S rRNA sequencing as species of NTM other than *M. fortuitum* and three as GPB, and not *M. fortuitum* as was initially identified by the GenoType Mycobacterium CM. The last isolate was identified by the GenoType Mycobacterium CM as *M. szulgai*, while the same was identified by 16S rRNA gene sequencing as *M. koreense*.

Table 3
Mycobacterium species identified from 16S rRNA sequencing

Species identified	Frequency	Percentage (%)
<i>M. fortuitum</i>	18	33.96
<i>M. novocastrense</i>	4	7.54
<i>M. flavescens</i>	3	5.66
<i>M. terrae</i>	4	7.54
<i>M. holsaticum</i>	3	5.66
<i>M. cosmeticum</i>	2	3.77
<i>M. virginense</i>	1	1.89
<i>M. intracellulare</i>	1	1.89
<i>M. mageritense</i>	1	1.89
<i>M. minnesotensis</i>	1	1.89
<i>M. duvalii</i>	1	1.89
<i>M. lehmannii</i>	1	1.89
<i>M. koreense</i>	1	1.89
Total	41/53	77.36%

Table 4
Comparison of GenoType Mycobacterium CM results and the 16S rRNA sequencing results

Sample ID	Species identified by: GenoType Mycobacterium CM	16S rRNA sequencing
JA004 Lungs	<i>M. fortuitum</i>	<i>M. fortuitum</i>
NA001 Lungs	<i>M. fortuitum</i>	<i>M. fortuitum</i>
NA002 Lungs	<i>M. fortuitum</i>	<i>M. fortuitum</i>
NA002 Spleen	<i>M. fortuitum</i>	<i>M. fortuitum</i>
NA005 Lungs	<i>M. fortuitum</i>	<i>M. fortuitum</i>
NA010 Lungs	<i>M. fortuitum</i>	<i>M. fortuitum</i>
NA011 Lungs	<i>M. szulgai</i>	<i>M. koreense</i>
NA013 Lungs	<i>M. fortuitum</i>	<i>M. fortuitum</i>
NA016 Lungs	<i>M. fortuitum</i>	<i>M. fortuitum</i>
NA019 Lungs	<i>M. fortuitum</i>	<i>Gordonia bronchialis</i>
NA020 Udder	<i>M. fortuitum</i>	<i>M. fortuitum</i>
NA021 Lungs	<i>M. fortuitum</i>	<i>M. cosmeticum</i>
NA028 Lungs	<i>M. fortuitum</i>	<i>M. novocastrense</i>
NA029 Liver	<i>M. fortuitum</i>	<i>M. fortuitum</i>
NA030 Liver Smooth	<i>M. fortuitum</i>	<i>Cellulosimicrobium cellulans</i>
NA030 Liver Yellow	<i>M. fortuitum</i>	<i>Cellulosimicrobium cellulans</i>
TA002 Liver	<i>M. fortuitum</i>	<i>M. mageritense</i>
HA007 Lymph Nodes	<i>M. fortuitum</i>	<i>M. minnesotensis</i>

3.5. Phylogenetic relationship between NTM species identified and the *M. bovis* reference strain

Although *M. bovis* was not isolated in the study, the reference strain sequence was added to the sequences of the identified NTM for a phylogenetic analysis to examine the relationship between the 13 different mycobacterial strains identified and the *M. bovis* reference strain.

The closest NTM species to the *M. bovis* reference strain in the phylogenetic tree was *M. intracellulare*. However, the most prevalent NTM species identified, *M. fortuitum*, was significantly diverse from the *M. bovis* reference strain (Figure 3).

3.6. Prevalence of multiple mycobacteria and mixed infections

The 41 NTM strains were isolated from 32 of the 50 cattle sampled, representing 64% NTM prevalence. The majority (21/41, 51.2%) of the NTM were isolated from the lungs, 10 were isolated from the liver, four from the lymph nodes, and two each were isolated from the spleen, udder, and intestines (Table 5). Five of the cattle (10%, 5/50) were found to have been infected with GPB. The combined infection rate, therefore, was 74% (37/50). Among the 37 infected cattle, 75.7% (28/37) were infected with a single NTM species, 10.8% (4/37) were infected with multiple (two to three) NTM species as co-infections, 5.4% (2/37) were co-infected with both NTM species and GPB species, while 8.1% (3/37) were infected with only GPB, as shown in Table 6.

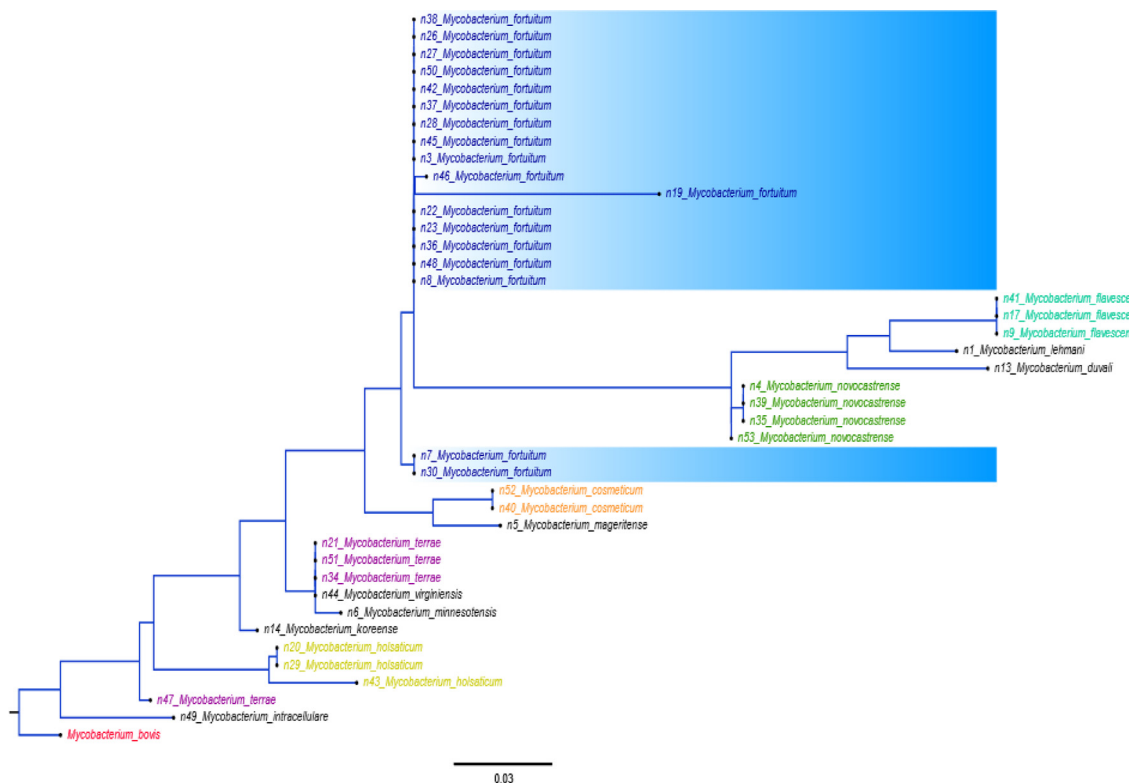


Figure 3. Phylogenetic tree showing the relationship between NTM strains and the *Mycobacterium bovis* reference strain. The phylogenetic tree was constructed using raxmlGUI software with the maximum likelihood algorithm and was aesthetically customized thereafter with FigTree v1.4.4.

Table 5
Distribution of non-tuberculous mycobacteria (NTM) based on tissue type

Tissue type	NTM strains
Lungs	21
Liver	10
Spleen	2
Lymph nodes	4
Intestines	2
Udder	2
Total NTM isolated	41

Table 6
Non-tuberculous mycobacteria (NTM) and gram-positive bacteria (GPB) infection dynamics among cattle

Infections	Number of cattle	Percentage
Single NTM infection	28	75.7%
Multiple NTM co-infection	4	10.8%
NTM and GPB co-infection	2	5.4%
Only GPB infection	3	8.1%

4. Discussion

Mycobacterium bovis is considered the main cause of bTB, while the neglected but opportunist NTM are considered a nuisance to the diagnosis of bTB. Therefore, in addition to the tuberculin made from killed *M. bovis*, the intradermal tuberculin skin test (TST) in live cattle uses another one from killed *Mycobacterium avium* (NTM) to prevent false-positive results. However, in countries like Ghana where bTB is thought to be epizootic, the identification of TB-like lesions in cattle during slaughter is often assumed to be indicative of bTB, especially if the lesions yield AFB. The objective of this study was to isolate and character-

ize mycobacterial species causing TB-like lesions in cattle in Ghana for epidemiological purposes and to guide therapy. This led to the sampling of 68 tissue samples from 50 cattle with characteristic TB-like lesions during post slaughter examinations.

The predominant cattle breed with TB-like lesions observed in this study was the WASH (58%, 29/50). This is because, more than 50% of the samples originated from the northern part of Ghana where the most preferred and predominant breed is WASH (Atiadeve et al., 2014), and this could account for the high frequency. The free grazing or nomadic system of raising cattle is practiced widely in Ghana. This system is known to expose cattle to infections of environmental pathogens such as NTM and *Nocardia* that cause granulomatous lesions in cattle (Michelet et al., 2018; Nuru et al., 2017).

Many of the NTM species were isolated from the lungs. This suggests that the primary route of infection was respiratory via aerosol inhalation. It has been established that although raising cattle by the free grazing or nomadic system is safer than zero grazing, this promotes close contact between them at night shelters, watering points, marketing yards, and at dipping tanks, thus exposing them to a high risk of pulmonary infections with mycobacteria (Ayele et al., 2004)

After DNA analysis and mycobacterial 16S rRNA gene sequencing, 77.4% (41/53) of the isolates were identified as mycobacterial strains, constituting 13 different species of NTM. The predominant strain was *M. fortuitum* (34.0%, 18/53), and no *M. bovis* or other MTBC species was identified. The current findings are consistent with reports from elsewhere, such as Ethiopia, where 96 cattle tissues with TB-like lesions were studied and neither *M. bovis* nor any of the MTBC species was identified. That study concluded that NTM was the major cause of TB-like lesions in the studied population (Nuru et al., 2017). A similar study in Kenya, however, isolated a few *M. bovis* ($n = 3$) from 218 bovine tissue specimens with TB-like lesions; the majority of the isolates were NTM, chief among them being *M. fortuitum* ($n = 12$) (Kurua et al., 2018). In another study in Tanzania, 55 NTM representing 16 different species were isolated from tissues of cattle, wildlife, and human sputum, with the ma-

majority ($n = 36$) isolated from cattle (Katale et al., 2014). The consistency of the current study findings with earlier reports elsewhere points to the fact that NTM are significantly implicated in TB-like lesions among cattle in Ghana, with *M. fortuitum* being the predominant strain.

Also in this study, six species of GPB were isolated, three of which (*Nocardia* sp, *Gordonia* sp, and *Dietszia* sp) have been reported elsewhere to cause TB-like lesions in cattle (Grist, 2008; Savini et al., 2012).

The isolation of NTM from TB-like lesions in cattle presents a major challenge to the diagnosis of bTB using the TST. This is because the bovine purified protein derivative (PPD) used for this test has been proven to cause cross-reactivity with *M. fortuitum* (the predominant species identified in this study) and other NTM species found in this study, resulting in false-positive reactions (Michel, 2008; Thacker et al., 2013). Also, some of the NTM species isolated in this study such as *M. fortuitum*, *M. intracellulare*, *M. novocastrense*, and *M. terrae* have been reported in human infections (Chen et al., 2009; Falkinham 3rd, 1996; Gharbi et al., 2019), especially among immunocompromised individuals such as those suffering from HIV/AIDS (Baird and Thomson, 2018; Kobayashi et al., 2016; Lee et al., 2011), lung diseases such as cystic fibrosis, chronic obstructive pulmonary disease (COPD), pulmonary tuberculosis, and lung cancer (Axson et al., 2019; Taiwo and Glassroth, 2010), surgical wound infections (Pinheiro et al., 2019; Singhal et al., 2013), and skin and soft tissue infections, gastrointestinal infections, and several other infections (Brode et al., 2017). In Ghana, speciation of mycobacterial isolates from the 2014 human population-based nationwide TB prevalence survey, revealed that more than 50% were NTM, with *M. fortuitum* being the most frequent (21.4%) (Addo et al., 2017). A similar study in Ghana on patients with pulmonary tuberculosis ($n = 1755$), isolated and identified 2.5% of total isolates as NTM species (Otchere et al., 2017).

Therefore, NTM infections in humans is not in doubt, although the source of transmission to humans is not clear. However, NTM are considered to be environmental mycobacteria mostly found in soil, water bodies, plants, and pastures (Hruska and Kaevska, 2012), and thus there is a high infection tendency in free grazing cattle as well as humans (Nuru et al., 2017). The outcomes of the current study provide the research evidence to tackle NTM infection in cattle in Ghana as an issue of both veterinary and medical importance based on the zoonotic consequences to human health. This suggestion is further strengthened by earlier work done by Malama and others in Zambia, where similar NTM were isolated from humans and animals at the interface of the same study area (Malama et al., 2013).

In conclusion, in cattle, NTM contribute significantly to TB-like lesions observed during slaughter examination and may be an important cause of zoonotic tuberculosis. A One Health surveillance of NTM in Ghana is recommended. Sampling from humans, animals, and environmental sources such as water and soil would provide insights into the zoonotic potential and clinical significance of these NTM.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical approval

Ethical approval was sought from the University of Ghana Institutional Animal Care and Use Committee (UG-IACUC), reference number UG-IACUC 003/19-20.

Author contributions

Conceptualization: Gloria Ivy Mensah. Data curation: Thomas Tingan, Yolanda Isabel Ayamdoo. Formal analysis: Thomas Tingan, Gloria Ivy Mensah, Lydia Mosi. Investigation: Thomas Tingan, Gloria Ivy Mensah, Edward Bensa Agyekum, Ivy Amanor, Samuel Ofori Addo, Mabel Sarpong Duah. Methodology: Gloria Ivy Mensah, Lydia Mosi. Project administration: Gloria Ivy Mensah, Lydia Mosi, Kennedy Kwasi Addo. Resources: Gloria Ivy Mensah, Lydia Mosi, Kennedy Kwasi Addo. Supervision: Gloria Ivy Mensah, Lydia Mosi. Validation: Gloria Ivy Mensah, Lydia Mosi. Writing – original draft: Thomas Tingan, Gloria Ivy Mensah. Writing – review and editing: Thomas Tingan, Gloria Ivy Mensah, Lydia Mosi, Kennedy Kwasi Addo.

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