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TUBERCULOSIS IN CATTLE AND HUMANS IN PLATEAU STATE, NIGERIA

A DISSERTATION SUBMITTED TO THE UNIVERSITY OF BRISTOL IN ACCORDANCE WITH THE REQUIREMENTS OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE FACULTY OF HEALTH SCIENCE

IJUGILA BITRUS FIORE

Abstract

Bovine tuberculosis (bTB) is a disease in cattle caused mainly by *Mycobacterium bovis*. Human tuberculosis (TB) is communicable among people and mainly caused by *Mycobacterium tuberculosis*. Humans are also susceptible to zoonotic tuberculosis caused by *M. bovis*. Both pathogens can be transmitted between animals and humans in either direction.

Retrospective records of routine post-mortem examination of cattle slaughtered in Jos Abattoir, Plateau State, Nigeria from 2001 to 2016 were analysed using logistic regression models to determine the prevalence of suspected TB lesions in slaughtered cattle and investigate possible associated risk factors. Similarly, retrospective laboratory records of TB tests on humans at the Jos University Teaching Hospital (JUTH) from 2001 to 2015 were analysed using logistic regression models to determine the prevalence TB in human patients presenting for testing.

Further studies were conducted to determine the current prevalence of suspected TB lesions in slaughtered cattle based on data obtained from detailed post-mortem examination of carcasses at the Jos Abattoir and to isolate and characterise mycobacterial agents from suspected TB lesions in these cattle slaughtered using real-time polymerase chain reaction.

Finally, knowledge attitudes and practices relating to zoonotic TB among livestock workers including abattoir workers and Fulani pastoralists in Plateau State were investigated by means of structured questionnaire surveys.

The retrospective observational study revealed that from 2001-2016, the overall prevalence of TB in slaughtered cattle examined routinely in the Jos abattoir was 4.0%. Multivariable logistic regression analysis of data on TB in these cattle revealed year, month, gender, age group, and location to be statistically significant (all p-values $< 10^{-9}$) factors in explaining TB prevalence.

The retrospective data on humans tested for TB at JUTH from 2001 to 2015 revealed an overall prevalence of TB of 14.2% and multivariable logistic regression analysis of revealed year, month, age group, and location to be to be statistically significant (all p-values $< 10^{-9}$) factors in explaining TB prevalence.

Detailed post-mortem examination of 500 carcasses of cattle slaughtered at the Jos Abattoir showed the true prevalence of TB in cattle to be almost five times higher (19.6%). Out of these 500 slaughtered cattle, 98 cattle had lesions suspected to be TB. Tissues with lesions were collected for DNA extraction and molecular analysis. Real-time PCR tests revealed 79 of the isolates to be *M. tuberculosis*. Two lesions contained 4 species (*Mycobacterium africanum, Mycobacterium caprae, Mycobacterium pinnipedii, Mycobacterium microti*) and seven isolates were unidentified mycobacteria. No *M. bovis* nor *M. bovis* BCG were found in any of the isolates.

Analysis of the questionnaire revealed that the majority of respondents were aware of TB, but there were knowledge gaps especially on the zoonotic nature of the disease. Age group, level of education and years of working with livestock were strong determinants of bTB knowledge, while occupation type, age group, and level of education were strong determinants of zoonotic TB preventive practices. The study also revealed risky practices including cohabiting with cattle, not using personal protective equipment, consumption of unpasteurised raw milk or "nunu", a yogurt-like milk product produced by Fulani pastoralists, and consumption of meat with TB lesions. These are indications of potential risks for zoonotic TB transmission.

In conclusion, the current study confirms that there is high prevalence of TB in humans and cattle in Plateau State and underestimation of the occurrence of TB in cattle. Identification of *M. tuberculosis* is an unusual and important finding in cattle and potentially of greater concern than the more common finding of *M. bovis* because it indicates a possibility of both human-to-cattle and cattle-to-cattle transmission of *M. tuberculosis* in Plateau State. The existence of risky practices could potentially expose individuals to zoonotic TB. These findings suggest collaborative efforts between the veterinary and public health sectors are required to design effective strategies to control the transmission of tuberculosis at the human-animal interface, including routine screening of livestock personnel in close contact with cattle.

Key. *M. bovis*, *M. tuberculosis*, reverse zoonosis, abattoir, cattle, Plateau State.

DECLARATION

I declare that the work in this dissertation was carried out in accordance with the Regulations of the University of Bristol. This work is original except where references were cited, and no part of the dissertation has been submitted for any academic award.

Ijugila Bitrus Fiore

June 2023

Dedication

This thesis is dedicated to my late mother Mrs Saraya M. Thliza. She taught me to persevere and prepared me to face the challenges with faith and humility. She was a constant source of inspiration to my life. Although she is not here to give me strength and support, I always feel her presence that used to urge me to strive to achieve my goals in life.

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And my daughter Saraya F. Fiore. You have made me stronger, better, and more fulfilled than I could ever imagined. I love you all to the moon and back.

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List of abbreviations

ACF	Autocorrelation function
ADA	Adenosine deaminase enzyme
AFB	Acid Fast Bacilli
AOR	Adjusted odd ratio
BC	Before Christ
BCG	Bacillus Calmette-Guèrin
Bp	base pair
bTB	bovine tuberculosis
CDC	Centres for disease control
CFSPH	Centre for Food Security and Public Health
CI	Confidence Interval
DEFRA	Department for Environment, Food & Rural Affairs
DNA	Deoxyribonucleic Acid
DR	Direct repeat
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and agricultural organization
HIV	Human Immunodeficiency Virus
HPLC	High performance liquid chromatography
IFN-γ	gamma interferon
IQR	Interquartile Range
JUTH	Jos University Teaching Hospital
LGA	Local Government Area
MIRU	Mycobacterium interspersed repetitive units
MPTR	Major polymorphic tandem repeat
MTC	Mycobacterium tuberculosis complex
NA	Nucleic acid
NTM	Non tuberculosis mycobacterium
NVRI	National Veterinary Research Institute
OIE	Office Internationale des Epizooties
OR	Odds Ratio
PACF	Partial autocorrelation function
PCR	Polymerase chain reaction
PPD	Purified Protein Derivate
PPD-A	Avium purified protein derivatives
PPD-B	Bovine purified protein derivatives
RAPD	Random amplified polymorphic DNA
RD	Region of Difference
REAL-TIME PCR	Real time polymerase chain reaction
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid

SICTT	Single Intradermal Comparative Tuberculin test
STL	Seasonal trend decomposition using Loess
TB	Tuberculosis
T*m*	Melting temperatures
TST	Tuberculin skin test
VNTR	Variable number tandem repeats
WHO	World health organization
ZN	Ziehl Neelson

CHAPTER ONE: OVERVIEW OF TUBERCULOSIS IN CATTLE AND HUMAN

1.1 Historical background of tuberculosis

Tuberculosis (TB) has been with us since ancient times. It has been hypothesized that the genus mycobacterium has been present for more than 15,000 years (Hayman, 1984). For many years, it was thought that *Mycobacterium tuberculosis* may have evolved from *Mycobacterium bovis*. However, findings by Brosch et al. (2002) on the new evolutionary scenario for *Mycobacterium tuberculosis* complex (MTC) contradicts this hypothesis.

TB was described in Italian writing more than 2000 BC and evidence of it in Neolithic people dates to 2400 BC where Egyptian mummies reveal skeletal deformities typical of TB (Morse et al., 1964; Salo et al., 1994; Zimmerman, 1979). Similar evidence was found from remains in Peru and Chile (Arriaza et al., 1995; Salo et al., 1994). The first written documents describing TB were from India and China, dating back to 3300 and 2300 years ago respectively (Brown, 1941; Cave and Demonstrator, 1939). Other written documents on TB are related to the Hebraism. TB was described in the books of Deuteronomy and Leviticus from the old testament of the Bible as schachepheth (an ancient Hebrew word) (Daniel and Daniel, 1999). In the Andean region in South America, the recovery and analysis of the remains of Peruvian mummies also revealed evidence of early TB including Pott's deformities. This suggest that TB was present even before the colonization by the first European pioneers in South America (Allison et al., 1973; Arriaza et al., 1995; Daniel, 2000a; Salo et al., 1994).

Until the end of the 19th century, TB was known as phthisis (Greek: phthinein, wasting) or consumption (Greek: to eat up or devour) because the bodies of sufferers seemed to waste as if consumed from within (Herzog, 1998; Lakhtakia, 2013). Johann Lukas Schönlein, a German naturalist and professor of medicine in the mid-19th century coined the term "tuberculosis" (Daniel, 2000b). Hippocrates described the clinical characteristics of patients with TB as

marked by fevers, sweats, sputa, and wasting (Cummings, 2007). Despite its frequency at the time, the cause of TB was still unknown (Herzog, 1998). In 1882 the famous scientist Robert Koch was able to identify, isolate and cultivate the tubercle bacillus responsible for TB (Gradmann, 2001, 2013). Since then, there have been several studies that were directed towards understanding the epidemiology of TB. In 1882, Robert Koch wrote that he believed that the same organism causes TB in both humans and animals and that one of the sources of TB transmission to humans is through inhalation of expectorant from TB sufferers, and the other is from TB infected animals. This attracted attention and led to increase interest in the prevention of infection by meat inspection and heat treatment of milk (milk pasteurization) (Palmer and Waters, 2011; Sakula, 1983).

TB in cattle was known as 'Pearl-diseases'. Many countries were concerned about this form of disease, it was believed that humans could become infected from eating meat from TB infected cattle and because of this, there were regulations banning its sale (Collins and Grange, 1983; Miller, 1989; Sakula, 1983; Salo et al., 1994).

1.2 Aetiology

TB is a chronic or acute bacterial disease caused by members of the MTC (Malama et al., 2013; Tamiru et al., 2013). Amongst the bacterial species that make up this group are *M. bovis* and *M. tuberculosis.* Normally, *M. bovis* is isolated from animals, particularly cattle, and *M. tuberculosis* is principally isolated from humans (Smith et al., 2006a). However, there have been instances where *M. bovis* has been isolated from humans (Zeweld, 2014) and *M. tuberculosis* isolated from cattle (Ameni et al., 2011).

1.3 Taxonomy of mycobacteria

Mycobacterium is a genus of the family Mycobacteriaceae, suborder Corynebacterineae, the order Actinomycetales under the phylum Actinobacteria. Under this genus are found several species. Mycobacteria can be classified into major groups: *Mycobacterium tuberculosis*

complex (MTC), *Mycobacterium leprae* (causes leprosy) and non-tuberculous mycobacteria (NTM) (Payeur, 2014; Porvaznik et al., 2017).

1.4 Mycobacterium tuberculosis complex

The *Mycobacterium tuberculosis* complex (MTC) is composed of several highly genetically related species with a similarity of 99.9% at the nucleotide level, and identical 16rRNA sequence (Behr, 2011; Brosch et al., 2002; Humblet et al., 2009).

Members of the MTC include *Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium bovis BCG, Mycobacterium caprae, Mycobacterium microti, Mycobacterium africanum, Mycobacterium microti, Mycobacterium canetti, Mycobacterium pinnipedii*, *Mycobacterium orygis Mycobacterium suricattae*, *Mycobacterium mungi*, and *Mycobacterium dassie* (Alexander et al., 2010; Coscolla and Gagneux, 2014; Orgeur and Brosch, 2018; van Ingen et al., 2012).

M. tuberculosis is the main pathogen that causes TB in the humans; it is also able to infect animals that have close contact with humans like the cattle (Gordon and Parish, 2018; LoBue et al., 2010; Ocepek et al., 2005). *M. canettii* and *M. africanum* can occasionally cause TB in humans especially those from Africa (de Jong et al., 2010; Fabre et al., 2010; Pfyffer et al., 1998). *M. bovis* is the major infectious agent that causes TB disease in cattle, other mammalian species, including humans, are also susceptible to infection by *M. bovis* (Delahay et al., 2002; Phillips et al., 2003). *M. caprae* causes TB in goats and humans. It has also been isolated from tuberculous lesions in cattle, pigs, deer, wild boars, camels, and bison (Kubica et al., 2003; Pate et al., 2006; Rodríguez et al., 2009; Rodríguez et al., 2011). *M. microti* causes TB in voles and other mammals like cats and llamas and it also causes diseases in human sporadically, especially in immunocompromised individual (Brosch et al., 2002; Niemann et al., 2000; van Soolingen et al., 1998; Xavier Emmanuel et al., 2007). *M. pinnipedii* primarily infects seals, although it is believed to cause TB in several species, including non-marine mammals and even

humans (Cousins et al., 1993a; Jurczynski et al., 2011; Kiers et al., 2008; Loeffler et al., 2014; Thompson et al., 1993). *M. orygis*, previously called the oryx bacillus have an unsettled host range (Huard et al., 2006; Smith et al., 2006b). They have been isolated from members of the Bovidae family, e.g oryxes, gazelles, antelope, deer, waterbuck and buffalo (Smith et al., 2006b; Gey van Pittius et al., 2012).

Furthermore, there are three members of the MTC that are closely related and have been isolated from Southern African mammals. This includes *M. suricattae*, which infect meerkats (*Suricata suricatta*) (Dippenaar et al., 2015; Parsons et al., 2013), *M. mungi*, which infects banded mongooses (*Mungos mungo*) (Alexander et al., 2010; Alexander et al., 2018), and *M. dassie* ("dassie bacillus") which infects rock hyraxes (*Procavia capensis*) (Clarke et al., 2016; Mostowy et al., 2004; Parsons et al., 2008).

1.5 Non-tuberculosis mycobacteria

Non-tuberculosis mycobacteria (NTM) are mycobacteria other than those belonging to the MTC, and they do not cause leprosy. They are referred to as atypical mycobacteria. Other synonyms of mycobacteria other than MTC are anonymous, unclassified, environmental, opportunistic, and saprophytic mycobacteria. There are more than 100 species of NTM. Examples of NTM species causing human and animal disease include *Mycobacterium avium, Mycobacterium bohemicam, Mycobacterium celatum, Mycobacterium chelonae, Mycobacterium conspicuum, Mycobacterium elephantis, Mycobacterium fortuitum, Mycobacterium genavense, Mycobacterium goodii, Mycobacterium habana, Mycobacterium haemophilum, Mycobacterium paratuberculosis, Mycobacterium abcessus*, *Mycobacterium asiaticum*, *Mycobacterium brasilienses*, *etc.*

NTM are free living organisms that are widespread in the natural and man-made environment. They inhabit a wide variety of environmental reservoirs such as soil, dust, natural occurring water, water source, water distribution systems, plumbing systems, food products, and animals

(Covert et al., 1999; Falkinham, 2011, 2013; Hutchison et al., 2019). NTM are usually saprophytic, but they can be opportunist and sometimes fatal especially in immunosuppressed individuals. They are responsible for a wide array of clinical manifestation such as pulmonary diseases (most frequent), lymphadenitis (common in children), skin or soft tissue diseases and they may also cause disseminated disease especially in HIV/AIDS patients (Al-Muhsen and Casanova, 2008; Griffith et al., 2007; Howard and Byrd, 2000; Wassilew et al., 2016; Weiss and Glassroth, 2012). Clinical presentation of pulmonary disease due to NTM may be similar to that caused by TB in humans and animals, and hence diagnosis of NTM is important as the treatment and control strategy for TB in humans and animals may differ from other mycobacteria (Katoch, 2004a). Failure to characterize acid-fast bacilli (AFB) positive NTM lung infections in humans has led to their misclassification and treatment errors for TB (Pokam and Asuquo, 2012). The implication is that NTM infected patients are wrongly managed with first line drugs meant for the treatment of pulmonary TB, thereby worsening the patient‘s condition and raising the risk of drug resistance (Koh and Kwon, 2004; Yim and Han, 2005). NTM has been known to cause disease in livestock characterized by the formation of TB-like lesions. During routine post-mortem examination in abattoirs, these TB-like lesions due to NTM in livestock may be incorrectly diagnosed to be bTB (Berg et al., 2009; Mamo et al., 2012; Shitaye et al., 2006b). Programs designed to detect TB routinely in cattle include tuberculin skin testing (TST). Studies have revealed that cattle pre-exposed to NTM may be a potential cause of false positive tuberculin test results because the compositions of mycobacteria are antigenically similar (Biet and Boschiroli, 2014; Katoch, 2004a; Nuru et al., 2017; Wachtman et al., 2011). This may have devastating and unnecessary consequence for the herd owner.

1.6 Morphology of mycobacteria

Mycobacteria are pleomorphic bacilli or cocci bacilli that are non-spore forming, and non-motile. The mycobacteria cell walls are very thick and consist of four layers. When viewed under the microscope, mycobacteria appear as straight or curved rod or club shaped (Figure 1.5). However, on media they are seen as cocci or rods about 0.2–0.6 μm wide and 1.0–10 μm long (Percival and Williams, 2014; Quinn et al., 1999). They may be seen singly, in pairs or in small bundles. Pathogenic mycobacteria are also known by their tendency to form characteristic cords (Grange, 1995; Percival and Williams, 2014). The formation of microscopic cords is linked to the virulence of the strain (Julian et al., 2010).

1.7 Alternative names for human tuberculosis

TB is referred to by different people in different ways. Some synonyms are: “Consumption” (because it seemed to consume people from within, and this is accompanied by a bloody cough, fever, pallor, and long relentless wasting); *Phthisis pulmonalis* (phthisis is the Greek name for ‘wasting disease,), ‘Scrofula’ (in adults) cervical tuberculous lymphadenitis and resulting in swollen cervical lymph nodes; *Lupus vulgaris* (TB of the skin); *tabes mesenterica* (TB of the abdomen); ‘wasting disease’, ‘white plague’ (because sufferers appear markedly pale); ‘King’s evil’ (it was believed that a king’s touch would heal Scrofula); ‘Pott disease’ is a term used for TB spondylitis, spinal TB or TB of the spine (Barberis et al., 2017; Murray et al., 2016).

Other names are “Miliary TB (disseminated TB) and ‘Schachepheth’, used by the ancient Hebrews to refer to wasting disease believed to have been TB. It is also named “Koch’s disease” after the scientist Robert Koch (Bhansali, 1977; Daniel, 2006; Matsuura and Nakatsu, 2017). The Hausa/Fulani people of Nigeria call it ‘Mashekari’ or ‘Tarin fuka’.

1.8 Pathogenesis

1.8.1 Pathogenesis of *Mycobacterium bovis* in Cattle

The pathogenesis of TB in cattle has not received the same level of attention as with TB in human and in many instances, the pathogenesis of TB in cattle have been drawn from studies of human TB or from experimental TB infections using small animal models (Neill et al., 2001).

The pathogenesis of TB in cattle may involve two stages: the formation of a primary complex and subsequent post-primary dissemination. The primary complex involves lesions at the point of entry and at the local lymph nodes. This mostly occurs in infections occurring through inhalation. Lesions are rarely observed when infection is via the alimentary canal, but tonsillar and interstitial ulcers are noticeable. Lesions are commonly seen at the pharyngeal or mesenteric lymph nodes (Radostits et al., 2003).

A primary focus develops within eight days of infection with the bacillus. About two weeks later, calcification of the lesions takes place. Shortly, thereafter granulation tissue, monocytes and plasma cells forms around the necrotic focus, leading to the formation of the pathognomonic “tubercle” (Radostits et al., 2003). According to Radostits et al. (2003), in 90-95% of *M. bovis* infected cattle, lesions are mostly confined to the regional lymph nodes of the head and caudal lobes of the lungs. Lesions may also be seen in the liver of calves, especially those fed with *M. bovis* infected milk.

After the primary complex comes post-primary dissemination. This occurs within weeks or years after initial infection and may appear as discrete nodular lesions in several organs, chronic organ TB or acute miliary TB which occurs when a large number of bacilli travel through the bloodstream and spread throughout the body and may lodge in organs causing minutes tubercules to form in the tissues of the organ affected (Radostits et al., 2003). Though lymph nodes are rarely involved in post primary dissemination, clinical signs vary between

infected animals depending on the site of infection. Persistent toxaemia weakens cattle and ultimately leads to their death. This is because the disease is always progressive (Radostits et al., 2003).

1.8.2 Pathogenesis of *Mycobacterium tuberculosis* in humans

The initial stage of infection is characterised by cell mediated immunity and formation of granulomas (a compact aggregate of inflammatory mononuclear cell). Activated T lymphocytes migrate to the site of infection, forming granulomas in which macrophages are activated and become capable of destroying most of the phagocytosed mycobacteria by the action of microbicidal molecules (hydrolytic enzymes, reactive oxygen intermediates and reactive nitrogen intermediates such as nitric oxide) in the phagosome (Hope and Villarreal-Ramos, 2008; Kaufmann, 2002; Thoen and Barletta, 2006). Cytotoxic T-cells release two protein substances called perforin and granulysin which bore into mycobacteria-infected cells, leading to necrosis (cell death) (Houben et al., 2006). At the centre of the region of infected cells, this process results in caseous necrosis (a soft and white cheese-like tissue) (Grosset, 2003).

When an infection occurs, the tubercle bacilli enter the body, in which they either become eliminated by the host immune system, or the host may remain asymptomatic with latent infection; or the host may develop primary and post-primary TB (Grange et al., 2011).

At the early stages of infection, 90% of patients infected with *M. tuberculosis* show no signs of infection, and out of this percentage, only 10% are likely to develop into active TB cases if left untreated. The mortality rate amongst cases of active TB infection can be up to 50% (Kumar et al., 2007).

Miliary TB (a widespread dissemination of *M. tuberculosis*) occurs when the bacillus finds its way into the circulatory system through a damaged infected tissue. It then spreads through the

body of its host and creates several small white tubercles as a result of multi-infection spots (Kim et al., 2003). The infection tends to increase and then later reduce. This is observed because after necrosis and tissue damage, fibrosis, and tissue repair sets in. Necrotic tissues fill the cavities, and the damaged tissues form scars. When the disease is active, infection can be transmitted when a patient coughs or sneezes. This is because many of the cavities are linked to the bronchi, and the material contains the living tubercle bacilli. Treatment with antibiotics can bring about healing. During the healing process, scar tissue replaces the damaged tissues (Grosset, 2003). The progression of TB disease can be summarized into 3 stages:

Stage I – Latent TB Infection (LTBI): The host remains asymptomatic with mild infection. In this phase, there are no signs or symptoms of an infection, and the host remains healthy. The tubercle bacilli change the chemical signature of the host body, and a chest x-ray would reveal a small scar on the lungs. The bacteria remain viable within the primary focus but are prevented from multiplying or spreading within the host. There is a constant battle between the host's immune system and the tubercle bacilli at the granuloma level. The body fights to prevent the bacteria from activation (multiplying in the body). It is only when there is an active TB case which is characterised with the presence of signs and symptom of TB, that the infection can spread amongst people, but in this LTBI phase, it cannot infect another host. It has been estimated that a third of the world's population have LTBI (Tufariello et al., 2003).

Stage II – Active TB: This usually happens within the first year or two after initial infection or when the immune system is weakened. During this stage, the tubercle bacilli multiply and spread within the body and the infection becomes symptomatic, indicating an active infection. This phase is more common in immunocompromised or malnourished individuals (Cardona, 2007).

Stage III – Secondary infection phase (reactivation): Dormant bacilli can be reactivated months or years after the initial infection and cause active and symptomatic TB disease. Post-

dormancy re-activations often occur in the lungs, being the main entry route of the bacteria, and rarely occurs in other organs (Glickman and Jacobs, 2001). The inert bacteria which multiply and spread within the host, are often hidden in previously formed granulomatous scars. The virulence of TB is affected by the rate at which the latent tubercle bacilli are reactivated in the host body (Basu and Galvani, 2009). This reactivation is often attributed to weak immune systems, especially amongst high-risk groups such as very old people, persons living with HIV/AIDS, malnourished individuals and those taking immune-suppressive drugs (Blanco et al., 2002).

1.9 Immunology of tuberculosis

A vital component of the immunological response to TB is the cell-mediated immunity (CMI). It is responsible for both the defence from the infection and the development of lesions in tissues (de la Rua-Domenech et al., 2006; de Martino et al., 2019; Domingo et al., 2014; Waters et al., 2014; Zuñiga et al., 2012). The CMI consist of both innate and the adaptive components and is driven by the intracellular nature of the bacillus (Ernst et al., 2007).

When the bacteria enter the respiratory tract, they pass through the mucociliary escalator to the alveolar space. Alveolar macrophages ingest the bacilli into a membrane bound vacuole termed the phagosome. This provides the first line of in vivo host defence mechanism (Hope and Villarreal-Ramos, 2008). The phagosome then undergoes sequential fusion events and acidification to acquire bactericidal and degrading characteristics by a process called maturation (Nguyen and Pieters, 2005). The mycobacteria can prevent lysosomal delivery by host-cell signalling manipulation, which reduces the response of cytokines involved in the host protection, while simultaneously adapting host cell metabolism to prompt the accumulation of lipid body (Nguyen and Pieters, 2005). It was long believed that mycobacteria live and multiply primarily inside the phagosomes and eventually destroying the phagocytes. However, recent

studies have described how two days after phagocytosis, *M. tuberculosis* is translocated from phago-lysosomes to the cytosol of myeloid cells (van der Wel et al., 2007).

Even though mycobacteria infected macrophages are not considered to function as the major antigen presenting cells (APC), they can stimulate an immune response through the release of TNF- α and chemokines (pro-inflammatory cytokines) which leads to other phagocytes and lymphocytes being recruited to the lungs (Arango Duque and Descoteaux, 2014; Hope and Villarreal-Ramos, 2008; Thoen and Barletta, 2006). In TB infection, dendritic cells most likely are the most potent APCs because of their unique capability to initiate the activation and differentiation of naïve T lymphocytes. The specialized intestinal epithelial cells (M cells) rapidly transport the mycobacteria to the lung tissues. The mycobacteria then penetrate the underlying lymphoid tissues and get ingested by dendritic cells which then move through the lymphatic vessels to regional lymph nodes, where the bacteria presentation to resident lymphocytes regulates immune responses (Hope and Villarreal-Ramos, 2008). The interaction of TB pathogens with dendritic cells leads to cell maturation and increases expression of surface molecules (such as the cell surface receptors, intracellular proteins, costimulatory molecules, cytokines, chemokines and their corresponding receptors, and proteases) involved in the activation and regulation of T-cell (Banchereau and Steinman, 1998; Hope et al., 2004). The secretion of cytokines such as TNF-α and IL-12 by the dendritic cell or macrophages infected with a TB pathogen triggers an adaptive cell mediated immune (CMI) response typically called the Th1-type immune response, which is characterized by the secretion of high levels of IFN-γ and IL-2 by Th1 CD4 T-cells (Hope et al., 2004; Thoen and Barletta, 2006).

When dendritic cells do not have a strong ability to kill ingested TB pathogens they are able to keep the numerous live bacilli in a nonreplicating state (Hope and Villarreal-Ramos, 2008). Dendritic cells may therefore constitute a reservoir for long-term survival of the pathogenic mycobacteria. Dendritic cells containing live mycobacteria may trigger the activation of T cells

more effectively or may be used by pathogens as a medium for more efficient spread (Hope and Villarreal-Ramos, 2008). Natural killer cells and γδ T-cells are necessary for the immune response against infections from mycobacteria. When these cells get activated by interacting with dendritic cells, they release IFN-γ, thereby contributing to the Th1-biased immune response (Hope and Villarreal-Ramos, 2008).

The host's attempt to contain and limit the TB infection is dependent on the initiation of a strong CMI response and the formation of a granuloma, in which the response of both CD4 and CD8 T-cells plays an important part. Therefore, the strength of the immune response determines how bacterial growth is contained, hence preventing active disease development, although mycobacteria infection may never be fully cleared.

When the balance between the host's defences and the pathogenic mycobacteria is tipped in favour of the pathogen, there will be spreading of the bacteria to uninfected macrophages that are recruited to the infected site, resulting to the occurrence of active TB disease and granuloma formation progresses further (Stewart et al., 2003). Formation of granulomas is a typical hallmark of TB. They are spherical structure with an organized collection of immune cells like macrophages, lymphocytes, and neutrophils. At the late stage of bTB infection, there is extensive necrosis of the granulomas which may ultimately lead to cavity formation. When these cavities rupture into the bronchi, bacteria can spread via aerosol to susceptible hosts (Thoen and Barletta, 2006).

The CMI response in TB infection is necessary for protection to occur. However, the CMI response can also contribute to TB immunopathogenesis. One of the functions of the Th2 immune response is to regulate the pro-inflammatory CMI response, elicited by IFN-γ producing cells (Hope and Villarreal-Ramos, 2008). It is also associated with an increased humoral immune reaction which is an adaptive response activated by CD4 Th2 cells and the release of cytokines including IL-4. It is believed that Th1-type immune responses decrease at

the late stage of TB disease. However, where there is increased bacterial load and progression of disease and pathology, the humoral Th2-type immune responses increase (Ashenafi et al., 2014; de la Rua-Domenech et al., 2006). The transition of Th1-type to a Th2-type immune response, as well as factors that trigger such transition are still not properly known. However, the CMI response is considered very vital in determining protection, while the humoral response is detrimental (Thoen and Barletta, 2006).

1.10 Epidemiology of tuberculosis

1.10.1 Host range of tubercle bacilli

Although the main host of *M. bovis* is cattle, other animals (domestic and wild) can be infected by the bacillus (Tschopp et al., 2011). African buffalo, badgers, bison, brushed tail opossums, elk, and kudu are maintenance hosts (Regassa, 2005). *M. bovis* however has a wide range of spill over hosts. These include animals such as sheep, goats, horses, pigs, dogs, cats, ferrets, camels, llamas, elk, elephants, deer, rhinoceroses, mink, coyotes, primates, otters, seals, sea, opossums, bears, hares, warthogs, foxes, raccoons, large cats (lions, tigers, leopards, cheetahs, and lynx) and many species of rodents (Gezahegne et al., 2012; Radostits et al., 2007; Tschopp et al., 2010). The aforementioned are animals known to be hosts of the mycobacteria. There might be other animals susceptible to *M. bovis* which are yet unknown. While birds are hosts to the related species *M. avium*, they are generally considered to be resistant to the *M. bovis*, although little is known about their susceptibility (Quinn et al., 1999; Tschopp et al., 2011). However, there have been reports of experimental infections in some avian species. In pigeons, infections occurred through oral or intra-tracheal inoculation, while in crows via intra-peritoneal inoculation (Admasu et al., 2014). Some avian species like mallard ducks proved resistant to experimental infections (Fitzgerald et al., 2005).

Humans are the natural reservoir for *M. tuberculosis* (Fitzgerald et al., 2015). No animal reservoir has been confirmed, although cases of TB in animals caused by *M. tuberculosis* have been reported (Adesokan et al., 2019; Mittal et al., 2014; Woldemariam et al., 2020).

Table 1.1 summarises the host range of the principal tuberculous agents of humans and animals. Factors that influence the rate at which the TB bacilli are transmitted are the route of transmission, the infectiousness of the host, the infective dose of the organism, the virulence of the tubercle bacilli strain, the length of exposure and the susceptibility of the host at risk (Biet et al., 2005).

Table 1. 1 Summary of principal tuberculous agents of human and animals

Species	*M. tuberculosis*	*M. bovis*
Human	P	P
Domestic Animals		
Canines – Dogs	P	P
Felines – Cats	0	P
Bovine – Cattle	P	P
Ovine – Sheep	0	0
Caprine –Goats	0	P
Porcine – Pigs	P	P
Equine – Horses	0	0
Avian in general – Poultry	0	0
Wildlife (M. bovis reservoirs)		
- Ungulates (eg. Buffalo, Bison)	0	P
- Cervids (e.g Deer, Lechwe, Elk, Wapiti…)	P	P
- Badgers, Brushtail, Possums, wild boars, other rodents)	P	P
Wildlife (*M. bovis* potential reservoirs)		
Others: Cloven hoof animals (e.g. the kudus, Ilama, some Deers, Giraffe, Ferrets, Oryx, Wild Goats, Impala, Yak, Eland, Wilde beest)	P	P
Wildlife (Carnivores & Scavengers – Wild Canines & Felines)		
Foxes, Tiger, Coyotes, Wolf, Lions, Cheetah, Leopard, Lynx, Racoon, Black Bear, Opossums, Bush Pig, Warthog)	P	P
Wildlife (Non-human primates and non-primates)		
- Monkeys/Apes, Gibbon, Mayotte, Baboons, Gorrillas,)	P	P
-Rhineceros, Hares…; Elephants,	P	P
- Birds: Psittacids…	0	0

Pathogenic Power: P= high, 0= Exceptional or rare

(Biet et al., 2005; Thoen et al., 2009)

1.10.2 Transmission of tuberculosis

Tubercle bacilli enter the body through many routes, with the alimentary and respiratory systems being the major routes, while the rarest are via congenital, cutaneous, and sexual transmission (Neill et al., 1994; Thoen and Bloom, 1995). Of the two major routes, the respiratory is the most important transmission route for infection amongst susceptible hosts that are in close contact. When mycobacteria are excreted through aerosol droplets (exhaled air), only a low infective dose of the microbe is required to establish an infection compared to other routes of transmission. In cattle and humans less than 10 bacilli and 10-20 million bacilli are the estimated doses needed to cause infection via respiratory and oral routes respectively (Cassidy, 2006; Goodchild and Clifton-Hadley, 2001; Morris et al., 1994; Neill et al., 2005; Palmer and Waters, 2006). Infection of humans and animals with TB results from animal-to-animal, animal-to-human, human-to-animal, and human-to-human transmission (Evans et al., 2007; Thoen and LoBue, 2007; Thoen et al., 2009).

1.10.2.1 Animal-to-animal *M. bovis* transmission

Transmission of TB from animal-to-animal normally occurs directly by inhalation of *M. bovis* contaminated sputum droplets exhaled from an infected animal (Drewe et al., 2013). Sometimes, cattle and other animals can acquire TB infection indirectly from contaminated pasture, water, tools, and mineral and feed licks. *M. bovis* is extremely resistant to extreme weather conditions and can survive months in the environment. Adequate moisture, low temperature, and diffuse sunlight are factors for the persistence of *M. bovis* in the environment (Barbier et al., 2017; Courtenay et al., 2006; Duffield and Young, 1985; Fine et al., 2011; Jackson et al., 1995; Palmer and Whipple, 2006; Tanner and Michel, 1999; Young et al., 2005). Nursing calves often become infected by ingesting *M. bovis* directly from the milk of TB infected cows (Delahay et al., 2007; Goodchild and Clifton-Hadley, 2006). Carnivores and scavengers can acquire TB by eating carcasses infected with *M. bovis* (Vicente and

Vercauteren, 2019). Infections can also be spread through cuts (Grange and Yates, 1994). The transcutaneous mode of infection in animals is mainly through bites from infected animals. This is common for badgers, cats and ferrets (Kaneene and Pfeiffer, 2006).

There have been outbreaks of TB due to *M. tuberculosis* in cattle and other domestic and wild animals but the possibility of animal-to-animal transmission of *M. tuberculosis* is not known (Villarreal-Ramos et al., 2018; Whelan et al., 2010). Although some findings have reported the attenuation of *M. tuberculosis* in bovine hosts, thereby reducing the probability of dissemination, this has not been proven but it has been suggested that *M. tuberculosis* pathogen may not be sustained in the animal population (Villarreal-Ramos et al., 2018; Whelan et al., 2010).

1.10.2.2 Zoonotic tuberculosis transmission

According to WHO (2020), a zoonosis is any infection or disease that is naturally transmissible from animals to humans. Zoonotic pathogens can spread to humans and from humans to animals through direct contact with animal or indirectly through food, water, or the environment (Figure 1.1). TB caused by *M. bovis* in humans is known as zoonotic TB (Olea-Popelka et al., 2017). The importance of *M. bovis* in contributing to TB infection in humans is not known especially in low-income countries. This could be due to limited diagnostic facilities and techniques to differentiate between *M. bovis* and *M. tuberculosis* (Abubakar, 2007; Müller et al., 2013; Olea-Popelka et al., 2017; Perez-Lago et al., 2014).

Cattle are very important and essential part of the lives of many African communities like the Fulani and Maasai pastoralist communities in Nigeria and Tanzania respectively where they represent wealth and are often at the centre of many traditional occasions (Ducrotoy et al., 2016; Ducrotoy et al., 2017; Olea-Popelka et al., 2017; van der Meer et al., 2015). Pastoralists and livestock workers who handle and are in close prolonged contact with infected cattle, such

as veterinary surgeons, farmers and abattoir workers, often are infected, particularly with pulmonary TB (Ayele et al., 2004). In urban areas, humans are more prone to extra-pulmonary TB. This is because most zoonotic TB infections are transmitted through ingestion of unpasteurized milk and raw meat which could be infected with *M. bovis* (Ayele et al., 2004; Daborn et al., 1996). Generally, people living with HIV/AIDS are more susceptible to bTB infections because of their suppressed immune systems (Ayele et al., 2004). *M. bovis* can be transmitted from cattle to humans through contact with infected carcasses. Crop farmers in Africa often use cattle manure to fertilize their farmland. This can potentially expose them to the mycobacteria which may be present in the faeces (Lawal and Babalola, 2014).

M. bovis, the primary pathogen that causes TB in cattle, has been reported to infect humans and there have also been reports of spillback of *M. bovis* infections from humans to cattle. According to Shitaye et al. (2007), when humans share dwellings with cattle, and consume milk that has not been pasteurized, there is a likelihood of *M. bovis* being transmitted back-and-forth between cattle and humans. The potential danger to animals of Ziehl–Neelsen (ZN) smear positive patients infected with *M. bovis* has been reported (Sjögren and Hillerdal, 1978; van Soolingen et al., 1994). There was a scenario reported by Fritsche et al. (2004) of TB in cattle in close contact with a 72 year old farmer infected with *M. bovis* at childhood. The strain isolated in both the cattle and the patient were identical. Some farm workers urinate habitually in their cowshed, and this may represent a source of infection for animals (Grange and Yates, 1994). This is because the genitourinary tract is a site of secondary dissemination and latency of mycobacteria in infected humans (Grandjean-Lapierre et al., 2018; Mateos Colino et al., 2003).

1.10.2.3 Reverse zoonosis of tuberculosis

M. tuberculosis is a pathogen in humans and does not have an animal reservoir. Animals that become infected with *M. tuberculosis* represent most probably accidental hosts. Cases of *M.*

tuberculosis in animals have been reported, especially in animals with close prolonged contact with humans (Alfonso et al., 2004; Cadmus et al., 2018; Montali et al., 2001; Oh et al., 2002; Romero et al., 2011; Zachariah et al., 2017). This kind of transmission has been referred to as 'reverse zoonosis' (any disease or infectious pathogen, whose normal reservoir is human, and is transmitted to other vertebrates). Reverse zoonotic TB has been implied for TB infection in animals caused by *M. tuberculosis* (Adesokan et al., 2019; Mittal et al., 2014; Woldemariam et al., 2020). Humans with active TB are believed to be the main source of *M. tuberculosis* infection in animals. *M. tuberculosis* has also been isolated in the milk of cows (Adesokan et al., 2019; Bezerra et al., 2015; BhanuRekha et al., 2015).

1.10.2.4 Human-to-human tuberculosis transmission

Humans are the main host and reservoir for *M. tuberculosis*, with human-to-human transmission occurring through the respiratory route (Click, 2015). The bacterium is usually transmitted through inhalation of the pathogen in droplet nuclei. The aerosol droplets are of 1-5µ diameter. The cough, shout, singing, sneeze or talking of a person carrying pulmonary or laryngeal TB disease can generate infectious droplet nuclei. These nuclei enter the mouth or nose and pass through the respiratory canal to the pulmonary alveoli of the lungs of a susceptible host (Houben et al., 2006; Kumar et al., 2007). Human-to-human transmissions of *M. bovis* are considered rare and are largely anecdotal. Also, the rate of this transmission mode of *M. bovis* among humans seems inconsequential in comparison to those of animal-to-human or animal-to-animal (O'Reilly and Daborn, 1995). Some findings reveal that there is a very low incidence of human-to-human transmission of *M. bovis* and the likelihood of an endogenous reactivation several years or decades after initial infection. This leads to the assumption that the pathogenicity of *M. bovis* could be less than that of *M. tuberculosis* in humans (Pasquali, 2004). However, human-to-human *M. bovis* transmission was confirmed between two immunocompromised patients from the same household in France by

spoligotyping, and also in a cluster of six persons in United Kingdom by DNA (Deoxyribonucleic Acid) fingerprinting and interviews with patients, which revealed common social links in all six persons and absence of any zoonotic link in all except one patient (Evans et al., 2007; Sunder et al., 2009; Van Soolingen, 2001). In the Netherlands in 1994, there was a reported case of human TB which was caused by *M. bovis* that was likely acquired through human-to-human transmission (van Soolingen et al., 1994). These human-to-human transmission of *M. bovis* infection could be linked to both host and environmental factors.

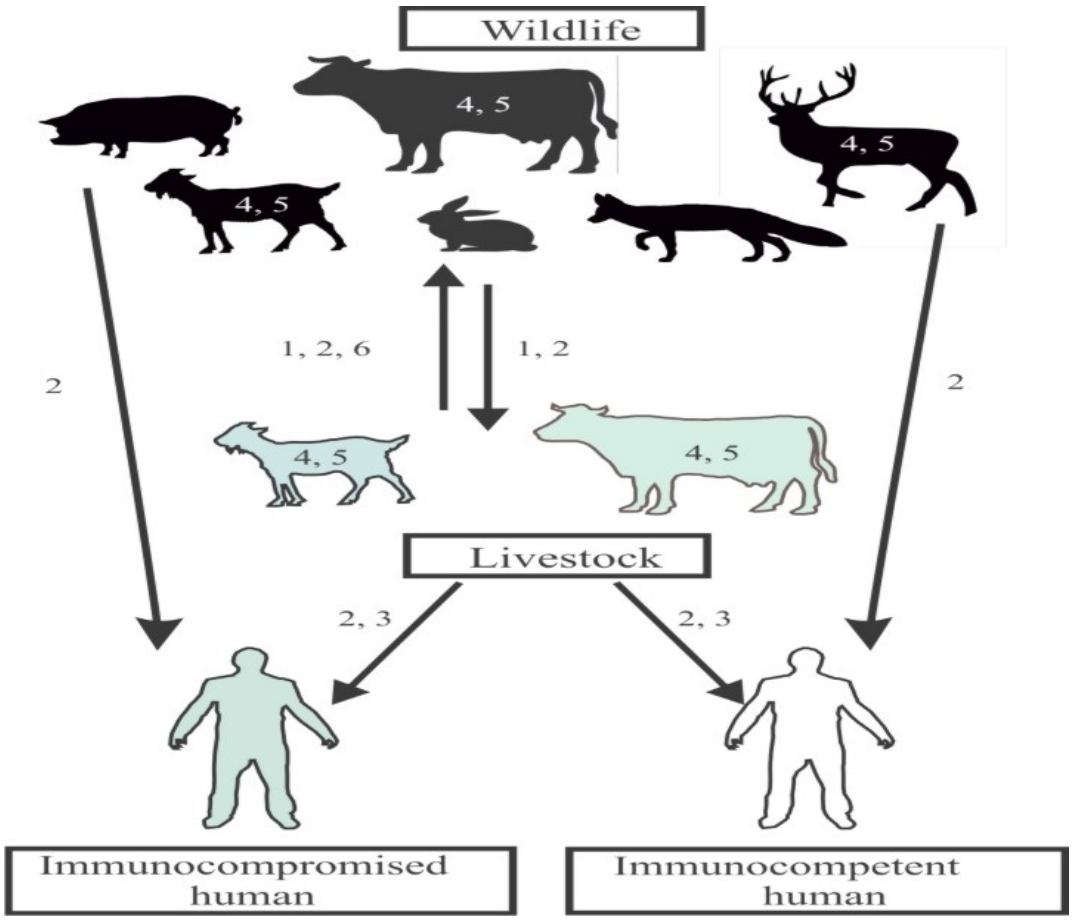

Figure 1. 1 Illustrated possible pathways of *Mycobacterium bovis* between the environment, wildlife, livestock, and humans

Source: Biet, F., Boschiroli, M. L., Thorel, M. F., & Guilloteau, L. A. (2005). Zoonotic aspects of *Mycobacterium bovis* and *Mycobacterium avium*-intracellulare complex (MAC). *Veterinary Research, 36*(3), 411-436

Legend

1. Infection by contaminated materials
2. Infection by aerosol
3. Infection by ingestion of livestock derived products
4. Vertical transmission
5. Horizontal transmission
6. Infection by predation

1.10.3 Epidemiology of tuberculosis globally

1.10.3.1 Epidemiology of bovine tuberculosis caused by *M. bovis*

Bovine TB, a chronic bacterial disease of animals is caused primarily by *M. bovis* of which cattle is considered to be the major reservoir, but other domestic and wild mammals and even humans can also be infected with *M. bovis* (Pollock et al., 2006). TB in humans due to *M. bovis* is referred to as zoonotic TB (Carruth et al., 2016). The burden of TB in humans due to *M. bovis* infection is underestimated especially in low-income countries where *M. bovis* is known to be endemic. This could be due to lack of funds, lack of awareness among health care providers and because the routine laboratory procedures used to diagnose human TB in these countries do not differentiate *M. tuberculosis* from *M. bovis* (Carruth et al., 2016; Cosivi et al., 1998b; Luciano and Roess, 2020; Olea-Popelka et al., 2017; Perez-Lago et al., 2014). Despite these limitations, the World Health Organization (WHO) in 2018, estimated that 143,000 new cases and 12,300 deaths globally, were due to TB in human caused by *M. bovis* infection (WHO, 2019b).

Bovine TB is found worldwide, although many high-income countries have controlled or eradicated bTB from their cattle population or reduced to very low levels through the implementation of control programs based on test-and-slaughter principles. However, significant pockets of bTB infection remain in wild animals (Corner, 2006; Kaneene et al., 2010). Bovine TB continues to be a major problem in countries which cannot afford such control programs. The free movement of cattle within and between countries and even continents facilitated the worldwide distribution and spread of bTB. The highest prevalence of bTB is in Africa and parts of Asia, but the disease is also found in the Americas and countries in Europe where the Eurasian badgers (*Meles meles*) are an important wildlife reservoir of TB (*M. bovis*) infection (Corner et al., 2011; OIE, 2021). Bovine TB is an important disease because of the risk it poses to public health and the loss of economic to the livestock industry

due to poor production performance, animal carcass condemnation, premature culling of animals and as a constraint for international trade of animals (Ejeh et al., 2014b; Kemal et al., 2019; Reviriego Gordejo and Vermeersch, 2006).

1.10.3.2 Epidemiology of human tuberculosis caused by *M. tuberculosis*

TB is a chronic infectious disease that affects humans (primarily caused by *M. tuberculosis*) and animals (primarily caused by other mycobacterium species) and it is responsible for a high death rate more especially in people infected with human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS). An increasing number of people are contracting TB because of their compromised immune system due to AIDS or immune-suppressive drugs. The World Health Organization (WHO) estimated persons infected with TB at approximately 10 million globally, of which 1.2 million died from TB among HIV negative people and an additional 251,000 people died from TB among HIV positive people in 2018 (Table 1.2) (WHO, 2019b). About a third of the world's current population has been infected with TB (Fig 1.2).

Apart from humans, *M. tuberculosis* infection has been reported sporadically to be isolated in animals, most frequently those that have close contact with humans (Ameni et al., 2011; Boulahbal et al., 1978; Hlokwe et al., 2017; Kassa et al., 2012; Michel et al., 2013; Michel et al., 2003; Paudel et al., 2019). This has been referred to as reverse zoonotic TB (Adesokan et al., 2019). Humans suffering from active TB are believed to be the most probable source of *M. tuberculosis* infection in animals (Ocepek et al., 2005; Steele, 1980).

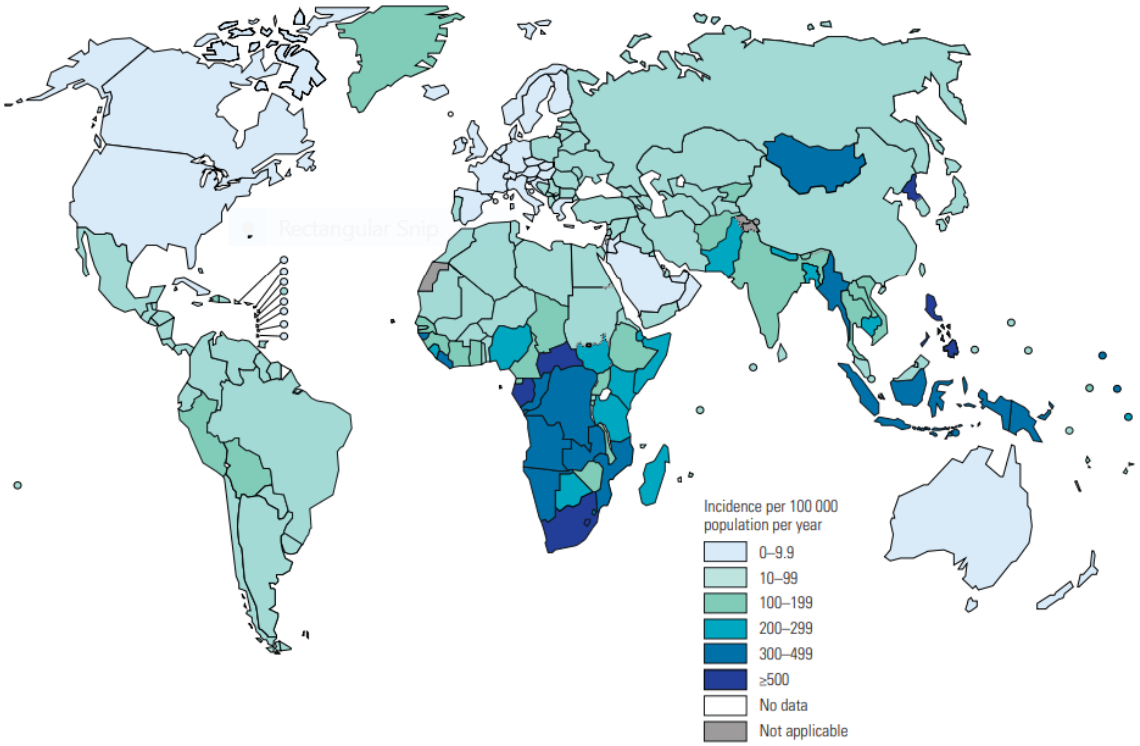

Figure 1. 2 Estimated TB incidence rates in humans in 2019

Source: World Health organization (WHO, 2020)

https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2022/tb-disease-burden/2-1-tb-incidence

1.10.4 Epidemiology of tuberculosis in Nigeria

1.10.4.1 Epidemiology of bovine tuberculosis in Nigeria

The cattle population in Nigeria has been estimated to be 20.5 million of which 90% of them are managed by Fulani pastoralists (Awogbade, 1979; Köster and de Wolff, 2012; Krauss et al., 2004; Tibi and Aphunu, 2010; World Bank, 2019). Cattle are the most commonly consumed animal protein source and contribute about 12.7% of the agricultural gross domestic product (GDP) in Nigeria (CBN, 1999).

The current map by WAHIS (2018) showing the burden of bTB across the world (Fig 1.4) does not reflect the true situation of bTB in Nigeria or for that matter, many other sub-Saharan

African countries which were shown correctly as having clinical disease present in the previous WAHIS 2015 map (Fig 1.3).

Nigeria among other countries has a high burden of bTB as shown in Figure 1.3 (WAHIS, 2015). Bovine TB was first reported in West Africa in 1913 in cattle from Cameroon. Due to the historical links Nigeria shares with Cameroon and the extensive transhumance movement of pastoralists with their cattle from Nigeria to other West African countries including Cameroon, cattle from Nigeria were assumed to have been infected with bTB from contact with infected cattle from Cameroon (Cadmus, 2019). Although there is limited epidemiological information on bTB in Nigeria, there are studies that have confirmed the endemicity of the disease in the country (Agbalaya et al., 2020; Akinseye et al., 2018; Ejeh et al., 2014a; Ejeh et al., 2014b; Ibrahim et al., 2010; Saidu et al., 2015; Tijani et al., 2020; Wekhe and Berepubo, 1989). From 1976 to 2007, the prevalence of bTB has increased from 2.5% to 14%. This statistic was acquired from the few surveys carried out in Nigeria over the past 30 years from tuberculin tests carried out by Alhaji (1976), Ayanwale (1984), Shehu (1988) and Abubakar (2007). Routine post-mortem examination of carcases is the only current bTB monitoring strategy in Nigeria which has been reported to be carried out irregularly or inadequately in some abattoirs, and it can only detect the more advanced forms of TB (Ahmad et al., 2018; Awah Ndukum et al., 2010; Carvalho et al., 2015; Edia-Asuke et al., 2014; Jaja et al., 2018). *M. bovis* is more often isolated from lung tissues and lymph nodes of cattle infected with TB in abattoirs/slaughterhouses in Nigeria. Other mycobacteria like *M. tuberculosis* and *M. africanus* have also been isolated (de Jong et al., 2010; Musawa et al., 2021). The lack of diagnostic facilities and technical know-how has made the study of outbreaks, the tracing of transmission routes and the determination of causative strains of the mycobacterium difficult (Abubakar, 2007). Other known bTB risk factors in Nigeria includes poor animal management,

unrestricted movement of livestock, weak veterinary services, and illiteracy (Abubakar et al., 2011a; Oloya et al., 2007; Saidu et al., 2015).

There is a need for proper and urgent development of Nigeria's health sector. Building scientific capacity in the country will assist in controlling bTB. Nigeria is being set back in its fight against bTB due to poor governance, lack of proper planning, accountability and commitment, high cost of sustainable testing and slaughter of infected animals, non-compensation of farmers, failure to carry out research on bTB and social unrest. This is due to political instability and ethnic wars, especially between the Fulani herders and local farmers (Abubakar et al., 2011a; Ayele et al., 2004).

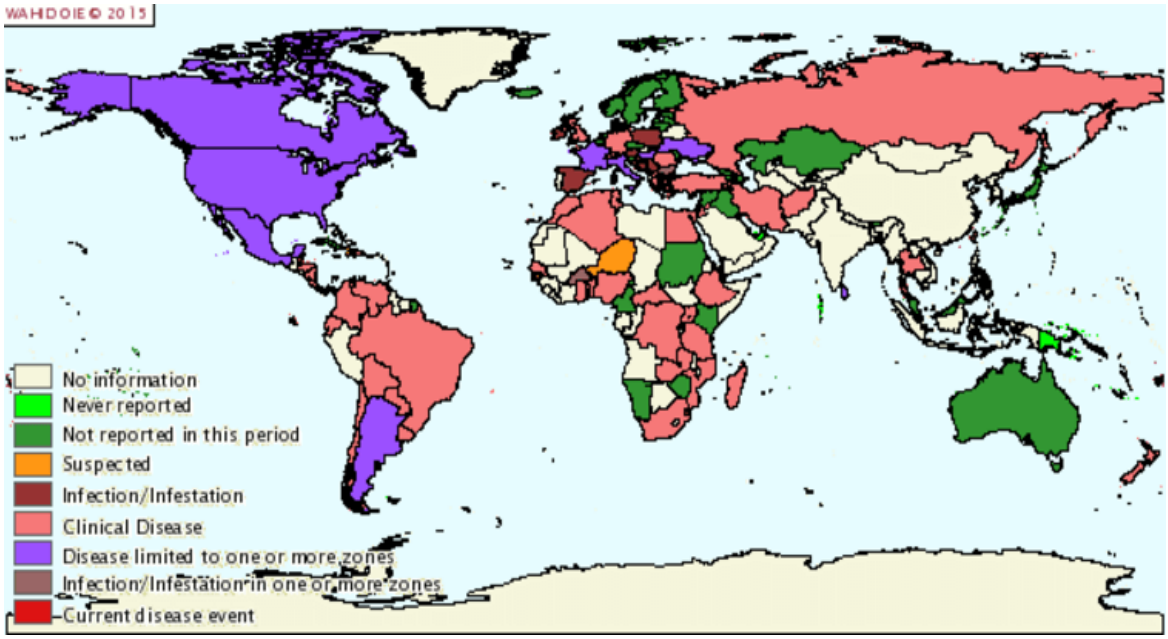

Figure 1. 3 Bovine tuberculosis across the world in 2015

Source: World Animal Health Information Database Interface (WAHIS, 2015)

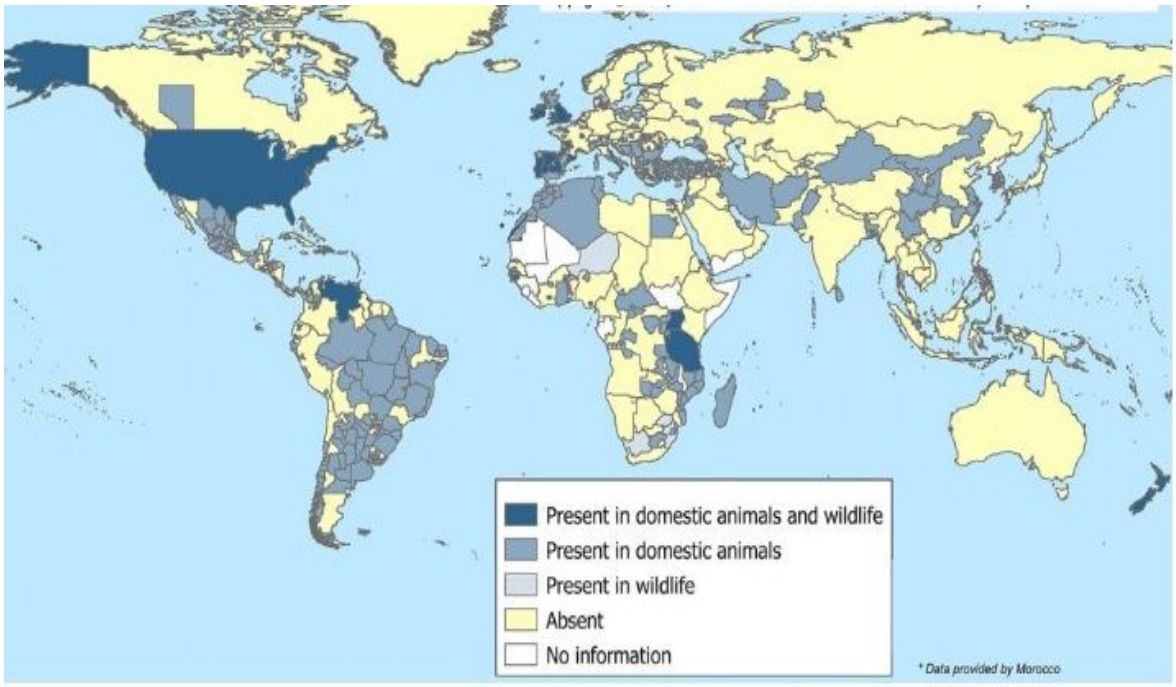

Figure 1. 4 Bovine tuberculosis across the world in 2018

Source: World Animal Health Information Database Interface (WAHIS, 2018)

1.10.4.2 Epidemiology of human tuberculosis in Nigeria

TB is one of the leading causes of morbidity and mortality in the country. More than 10% of all deaths in Nigeria are caused by TB (Aliyu and Amadu, 2017; WHO, 2019b). Nigeria is listed among the 30 countries with the highest TB burden worldwide and it has the highest number of TB infections within the African region (WHO, 2019b). It is also one among the eight countries contributing 87% of new TB cases globally (WHO, 2019a). It accounted for an estimated number of 429 TB cases per 100,000 population in 2018 and 155,000 deaths within the same period (WHO, 2019b). These estimates may be lower than the actual number of TB cases because of underreporting; Nigeria has one of the lowest global TB case detection rates at 15% in 2018 (WHO, 2019b). Nigeria also has a high burden of HIV/AIDS. In fact, it has the second largest population of people living with HIV/AIDS in the world. In 2019, it was estimated that 1.8 million people were living with HIV/AIDS in Nigeria (The World Factbook, 2019). The TB situation in Nigeria has been worsened by the prevalence of HIV/AIDS.

M. bovis, the primary cause of TB in cattle has also been identified and isolated in human sputum in Nigeria, especially from Fulani herdsmen, with or without clinical signs of the disease (Abubakar, 2007; Cadmus et al., 2006; Cadmus et al., 2007). The rate of zoonotic TB caused by *M. bovis* in Nigeria is unknown, although it is estimated that in some developing countries up to 10% of human TB is due to *M. bovis* (Khan et al., 2019). The country also lacks data on the rate of isolation of *M. bovis* from patients with HIV/AIDS. This only adds to the fact that the epidemiology of bTB as well as its health significance to the public are poorly understood (Cadmus et al., 2004). What is known is that consumption of unpasteurised milk by humans, and persistent proximity to animals are the primary sources of infection. There are studies that have reported *M. bovis* isolated in both raw and sour milk found in local markets in Nigeria (Abubakar, 2007; Alhaji, 1976; Okolo, 1992; Shehu, 1988). Unfortunately, in Nigeria most rural and some urban dwellers still readily ingest *M. bovis*-infected unpasteurised raw and sour milk known as "nunu" (Ayele et al., 2004).

1.11 Control of human tuberculosis in Nigeria

The Federal Government of Nigeria established the National Tuberculosis and Leprosy Control Programme (NTBLCP) in 1988 and in 1993 they adopted the directly observed treatment short course (DOTS) strategy which was recommended by the World Health Organization (WHO) as the global response to the rising TB epidemic (WHO, 1994a). In 2006, the DOTS strategy was replaced by the Stop TB Partnership strategy (2006–2015) to address challenges encountered with the implementation of the DOTS strategy and important control components that were previously left out, such as the rising HIV infections and multidrug resistant TB (MDR-TB), strengthening the health system for TB care, empowering communities, and people with TB through partnership, and promoting TB research. In line with the sustainable development goals, Nigeria embraced the End TB Partnership initiative whose ultimate target is to eliminate TB as a public health problem by the year 2035 globally (FMOHN, 2014; WHO,

2015). This initiative has three main pillars: 1. integrated, patient-centred care and prevention; 2. bold policies and supportive systems; and 3. intensified research and innovation (WHO, 2015).

Despite the implementation of current global TB control initiatives, TB remains a public health challenge in Nigeria. The effective control of TB in humans in Nigeria has been affected by many factors including poor policy implementation, lack of TB-specific legislation, exclusion of TB from health insurance, the HIV epidemic, widespread anti-TB drug resistance, weak, underfunded health systems that are unable to support efficient scale-up of TB services, lack of adequate TB diagnostic capacity poverty, overcrowding, under nutrition, lack of education, lack of knowledge about TB, stigmatisation and discrimination, insufficient human resources, noncompliance with TB patient monitoring requirements, weak regulatory framework, patient treatment interruption, poor adherence to TB treatment guidelines and limited pragmatic policy actions to tackle emerging risk factors for TB at the population level (Adejumo et al., 2016a; Adejumo et al., 2016b; Adejumo et al., 2020; Adejumo et al., 2016c; Adejumo et al., 2015; Alao et al., 2020; Bigwan et al., 2013; FMOH, 2014; Hassan et al., 2017; Ogbuabor and Onwujekwe, 2019; Tobin-West and Isodje, 2016).

In Nigeria, the routine laboratory diagnosis for TB is based on the relatively insensitive smear microscopy. Although cheap and easy to perform, microscopy cannot identify the species of mycobacterium involved thereby making it difficult to study TB outbreaks and trace infections to their origins (Cadmus et al., 2019). Another setback to the control of TB in Nigeria is that many people such as pastoralists, abattoir workers and veterinarians are in close contact with cattle and that many of the general public indulge in practices such as consuming unpasteurized milk or raw infected meat which potentially exposes them to zoonotic TB (Cadmus et al., 2019). The current national TB control initiatives do not make any provision for zoonotic TB transmission, thereby compromising effectiveness of control (Cadmus, 2019).

Table 1. 2 Estimated epidemiological burden of TB in 2018 for 30 high TB burden countries, WHO regions and globally. Numbers in thousands[a]

COUNTRIES	POPULATION	TOTAL TB INCIDENCE		HIV-POSITIVE TB INCIDENCE		HIV-NEGATIVE TB MORTALITY		HIV-POSITIVE TB MORTALITY[b]	
		BEST ESTIMATE	UNCERTAINTY INTERVAL	BEST ESTIMATE	UNCERTAINTY INTERVAL	BEST ESTIMATE	UNCERTAINTY INTERVAL	BEST ESTIMATE	UNCERTAINTY INTERVAL
Angola	31 000	109	71-156	11	6.8-15	19	11-28	3.7	2.4-5.3
Bangladesh	161 000	357	260-469	0.73	0.36-1.2	47	30-67	0.19	0.094-0.32
Brazil	209 000	95	81-110	11	9.3-13	4.8	4.6-5.0	1.9	1.4-2.4
Cambodia	16 000	49	27-77	1.1	0.59-1.7	3.0	1.9-4.3	0.38	0.21-0.60
Central African Republic	5 000	25	16-36	6.6	4.2-9.4	4.8	2.8-7.3	3.1	2.0-4.5
China	1 430 000	866	740-1 000	18	9.8-28	37	34-41	2.4	1.2-4.0
Congo	5 000	20	12-18	5.7	2.9-9.4	3.0	1.7-4.6	2.3	1.2-3.8
DPR Korea	26 000	131	114-149	0.22	0.12-0.36	20	14-27	0.068	0.035-0.11
DR Congo	84 000	270	175-385	31	9.4-65	43	25-65	10	3.2-22
Ethiopia	109 000	165	116-223	7.6	5.3-10	24	15-36	2.2	1.5-3.0
India[c]	1 350 000	2 690	1 840-3 700	92	63-126	440	408-472	9.7	5.7-15
Indonesia	268 000	845	770-923	21	8.9-38	93	87-99	5.3	2.1-9.8
Kenya	51 000	150	92-222	40	25-60	19	11-30	13	8.1-20
Lesotho[d]	2 000	13	8.3-18	8.4	5.4-12	0.95	0.56-1.4	3.3	2.1-4.7
Liberia	5 000	15	9.6-21	2.6	1.7-3.7	2.7	1.6-4.1	1.0	0.67-1.5
Mozambique[d]	29 000	162	105-232	58	38-83	21	13-32	22	14-31
Myanmar	54 000	181	119-256	15	10-22	21	12-31	3.7	2.5-5.2
Namibia	2 000	13	9.2-17	4.5	3.2-5.9	1.6	1.0-2.3	1.5	1.1-2.1
Nigeria	196 000	429	280-609	53	34-75	125	73-192	32	20-47
Pakistan	212 000	562	399-754	3.8	2.5-5.4	43	35-52	1.3	0.83-1.8
Papua New Guinea	9 000	37	30-45	2.7	2.2-3.3	4.5	3.0-6.2	0.25	0.10-0.45

Philippines	107 000	591	332-924	10	4.1-19	26	22-30	0.60	<0.01-4.2
Russian Federation	146 000	79	51-112	16	10-22	9.2	8.3-10	1.3	0.57-2.2
Sierra Leone	8 000	23	15-33	2.9	1.9-4.2	2.6	1.5-3.9	0.70	0.44-1.0
South Africa[d]	58 000	301	215-400	177	127-235	21	20-23	42	30-57
Thailand	69 000	106	81-136	11	8.2-14	9.2	6.9-12	2.3	1.7-3.0
UR Tanzania	56 000	142	67-245	40	19-69	22	10-40	16	7.8-27
Viet Nam	96 000	174	111-251	6.0	3.8-8.6	11	6.7-15	2.2	1.4-3.2
Zambia	17 000	60	39-86	36	23-51	4.8	2.9-7.3	13	8.3-19
Zimbabwe	14 000	30	22-39	19	14-24	1.1	0.69-1.7	3.5	2.4-4.8
High TB burden continents	**4 830 000**	**8 690**	**7 670-9 770**	**709**	**626-797**	**1 080**	**1 010-1 170**	**201**	**175-229**
Africa	1 060 000	2 450	2 190-2 730	615	539-697	397	331-468	211	184-239
The Americas	1 000 000	289	268-310	29	27-31	17	16-19	5.9	5.2-6.6
Eastern Mediterranean	704 000	810	639-1 000	6.9	5.3-8.8	77	66-89	2.2	1.6-2.8
Europe	927 000	259	225-296	30	23-37	23	22-24	4.4	3.3-5.6
South-East Asia	1 980 000	4 370	3 480-5 370	140	107-178	637	598-677	21	16-28
Western Pacific	1 920 000	1 840	1 520-2 180	41	30-54	90	83-98	6.5	4.9-8.4
Global	**7 600 000**	**10 000**	**8 990-11 100**	**862**	**776-952**	**1 240**	**1 160-1 320**	**251**	**224-280**

[a] Numbers shown to two significant figures if under 100 and to three significant figures otherwise

[b] Deaths among HIV-positive TB cases are classified as HIV deaths according to ICD-10

[c] Estimates of TB incidence and mortality for India are interim, pending results from the national TB prevalence survey planned for 2019/2020

[d] Estimates of TB incidence and mortality for Lesotho, Mozambique and South Africa will be reviewed after results from their respective national TB prevalence surveys are available

Source: WHO (2019b) https://apps.who.int/iris/bitstream/handle/10665/329368/9789241565714-en

1.12 Diagnosis of tuberculosis

1.12.1 Clinical examination

Due to the chronic nature of the disease, it is not easy to diagnose TB in cattle clinically. This is because the symptoms are numerous and depend on location of the infection (Radostits et al., 2007; Tsegayea et al., 2010). However, the presence of enlarged superficial lymph nodes is a helpful sign in diagnosing the disease, especially when the lungs are involved. Intermittent coughing, severe weight loss or emaciation despite proper feeding are characteristic signs of the disease. In the initial phase of infection there are usually no clinical signs and development of the disease depends on the route of infection (Radostits et al., 1994; Thoen and Bloom, 1995).

1.12.2 Post-mortem examination

The post-mortem examination of carcasses and meat of slaughtered cattle in abattoirs can be used to diagnose TB. The presence of abscess with yellow pus or caseous necrosis in tissues which may be gritty upon incision could indicate TB (FAO, 1994; Grist, 2008). In many low-income countries, observation of TB lesions through post-mortem examination is a major mode of detecting bTB (Asseged et al., 2004; Shitaye et al., 2006a). Some infected carcasses may not present lesions during post-mortem examination and when lesions are grossly visible, this implies the disease is already at an advanced stage. Lesions that are small may be missed and some non-tuberculous granulomas may be indistinguishable macroscopically from tuberculous granulomas. The sensitivity of post-mortem examination is affected by the method employed and the organs examined (Corner, 1994; Shitaye et al., 2006a).

1.12.3 Direct detection methods

1.12.3.1 Culture of mycobacteria

Culture is considered the "gold standard" and definitive test for the confirmation of TB (Cavalhiero et al., 2020). The isolation and cultivation of *M. bovis* and *M. tuberculosis* can only be carried out in a category 3 biosafety laboratory because they are hazardous agents. Specimens are processed for culture. First, the sensitivity of the culture is enhanced by homogenizing the tissue by pounding with a mortar and pestle or by grinding with a blender or stomacher. Next, it is decontaminated using a detergent like 0.375 – 0.755% hexadecyl pyridinium chloride (HPC), an acid like 5% oxalic acid, or an alkali (2 – 4% sodium hydroxide). The mixture (either acid or alkali) should be kept at room temperature and then shaken for 10 – 15 minutes. The pH is then balanced by neutralizing the mixture (Chernecky and Berger, 2013). The suspension is centrifuged and then the supernatant is discarded. The resulting sediment can be used for culture and microscopy. If the sediment is to be used for primary isolation, it needs to be inoculated on a collection of sloping egg-based media known as "slopes", containing either pyruvate alone or pyruvate and glycerol. A sloping media is used because it provides a greater surface area for the bacteria to grow. Examples of these egg-based media are Coletsos base, Kirchner, Lowenstein-Jensen, and Stonebrink. The most popular egg-based solid media is Lowenstein-Jensen medium which contains glycerol that support the growth of *M. tuberculosis* but inhibits the growth of *M. bovis*, while pyruvate containing media without glycerol enhance the growth of *M. bovis*. The isolation can also take place using an agar-based medium like Middlebrook or blood-based agar (Moore et al., 2006).

The culture needs to be incubated at 37ºC, either in the presence or absence of carbon dioxide. Incubation should be for at least a minimum of 8 weeks and preferably last 10-12 weeks. Desiccation should be avoided. To do so, the media must be closed in airtight tubes. When there is visible physical growth on the medium slopes (Figure 1.5), smears are prepared and

stained with ZN stain. *M. bovis* takes 3 to 6 weeks to begin to grow. The variation in time is dependent on the media used (OIE, 2009).

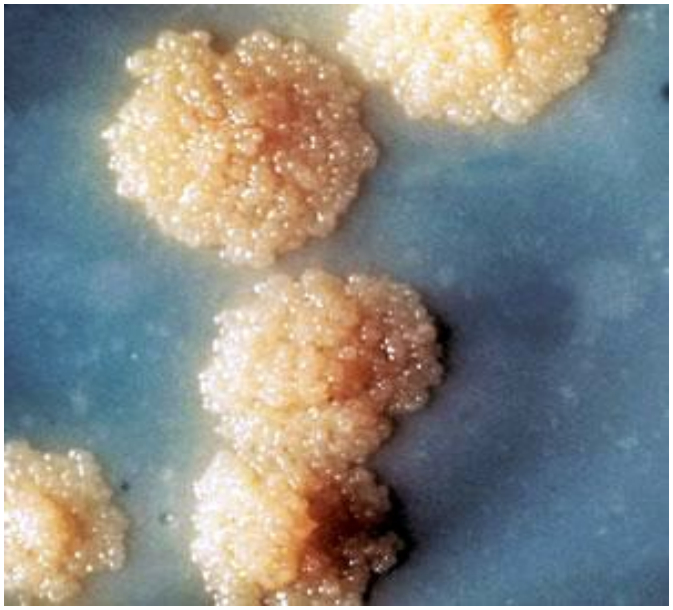

Figure 1. 5 Image of *M. tuberculosis* colonies on growth medium

Source: Centres for Disease Control and Prevention (CDC)
https://phil.cdc.gov/Details.aspx?pid=4428

There are now faster methods using automated cell culture systems. They include the BACTEC 9000, the MB/BacT, and the Mycobacterial Growth Indicator Tube (MGIT) (Drobniewski et al., 2003). A method considered more accurate and faster is the microscopic observation drug susceptibility assay culture (Moore et al., 2006).

The disadvantage of using culture methods in the diagnosis of TB is that it is an extremely slow procedure which may take months to complete and the sensitivity is not 100%; false-negative culture results can occur (Duffield et al., 1989). However, culture has the advantages of being more sensitive than microscopy and it has the ability to detect low mycobacterial loads (Della, 2004).

1.12.3.2 Microscopy/Histopathology

Mycobacteria can be demonstrated microscopically by direct smears from prepared tissue materials and clinical samples. These are stained with ZN stain and observed with an ordinary light microscope for the presence of AFB (Reynolds et al., 2009). Following application of carbol fuchsin stain and heating to promote entry of the stain into the cell wall, acid-fast bacteria can resist decolorization with acidified organic solvents used in the process due to their high lipid content which is 20-40% of the dry cell weight. Under the microscope, they appear as red or pink, colloidal, or bacillary cells 1-3 microns in length occurring singly or in clumps (Figure 1.6) (Percival and Williams, 2014; Scanlar, 1988). The ZN stain is a rapid method for detecting acid fast bacilli (The staining procedure itself takes minutes as does the microscopic examination). The ZN sputum smear is the standard method for diagnosing human TB in many low-income countries because it is cheap and has a high positive predictive value for TB. However, its limitation is that it has a low detection rate (i.e. diagnostic sensitivity) of about 0-20% in human sputum samples and it is not able to detect bacilli that are fewer than either 10,000 per slide, or 10,000 per ml of specimen 10,000 (Trusov et al., 2009; Ulrichs et al., 2005; Yeager et al., 1967).

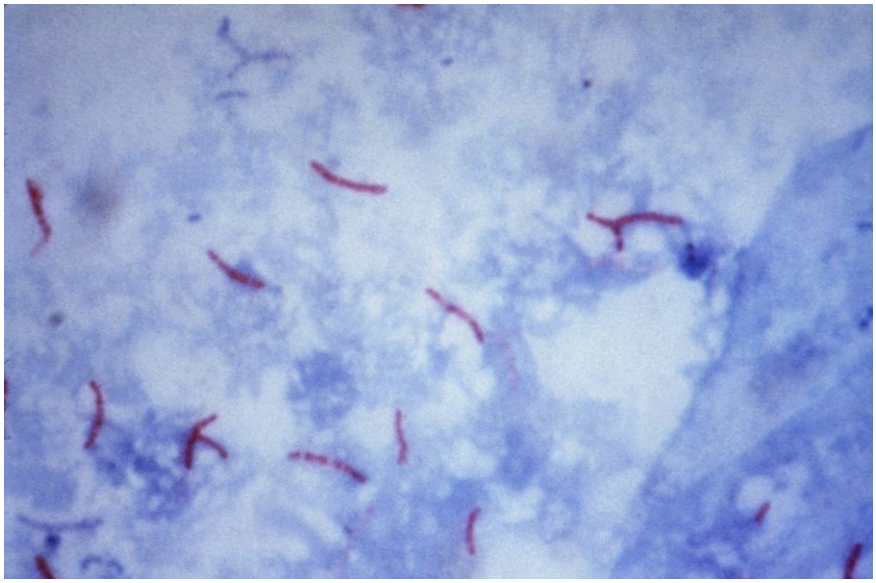

Figure 1. 6 *M. tuberculosis* (red bacilli) magnification of 1000X microscopy visualization using the Ziehl Neelsen stain for acid fast micro-organisms

Source: Centres for Disease Control and Prevention (CDC)
https://phil.cdc.gov/details.aspx?pid=5789

During necropsy of cattle, samples of tissues with lesions suspected to be bTB are collected and examined for histopathological lesions characteristic of mycobacteria infection, such as caseous necrosis, epithelioid cells, mineralization, multinucleated giant cells, and macrophages. However, the presence of these characteristic lesions is only a presumptive diagnosis of the disease condition (OIE, 2009; Schiller et al., 2010).

1.12.4 Indirect test

1.12.4.1 The single intradermal comparative tuberculin test

In live cattle, the single intradermal comparative tuberculin test (SICTT) (with bovine and avian tuberculin) is considered the standard diagnostic test and is used in eradication campaigns for the detection of infected animals. It has the advantage of being able to distinguish *M. bovis* infected animals from those sensitized to tuberculin due to infection with other species of mycobacteria or any similar genus (OIE, 2009). The thickness of the skin is measured, and then

the tuberculin is injected intradermally either at the mid-neck region or caudal fold of the tail. Seventy-two hours afterwards, any swelling at the injection area is measured. A purified protein derivative (PPD) product has a wider acceptability than heat-concentrated synthetic medium tuberculin. This is because the PPD product is readily available in commercial quantities and it has higher specificity and simpler standardization (OIE, 2009).

1.12.4.2 The single intradermal test

The single intradermal test (SID) involves an intradermal injection of a single dose of 0.1ml PPD into a point about 7cm to the centre of the caudal fold distal to the tail base. Results of the test are read by visual observation and by palpation of any swelling at the injection point 72 hours after the injection. Animals that present no reaction at the injection site are classified as "negative". When there is a positive reaction at the site, but the history of the animal does not show previous signs of TB, the animal is classified as "suspect". However, if there is a positive reaction at the site of injection and the medical history of the animal suggests TB, the animal is classified as a "reactor" and are immediately isolated from the rest of the herd until they are slaughtered. Animals classified as suspects are either slaughtered with permission or retested withing 10 days or otherwise not before 60 days after commencement of the caudal fold test (Hirsh and Zee, 1999; Monaghan et al., 1994; Radostits et al., 2007).

The disadvantages of the SID test are its lack of specificity and its failure to detect cases of minimal sensitivity, of early infections, in cows that recently calved, and in some cattle in unresponsive state due to immunosuppression (Andrews, 2003; Hirsh and Zee, 1999; Radostits et al., 2007).

1.12.4.3 Lymphocyte proliferation assay

The lymphocyte proliferation assay can be carried out on whole blood (Buddle, 2001) or on purified lymphocytes obtained from samples of peripheral blood (Griffin et al., 1993).

Tuberculin PPD from *M. bovis* (PPD-B) and Tuberculin PPD from *M. avium* (PPD-A) both react to peripheral lymphocytes. This assay can differentiate the reactivity of the two PPDs. An animal may be exposed to other species of mycobacteria. This test can isolate the reactions of lymphocytes to antigens of other species of mycobacteria that are not pathogenic, thus increasing the test's specificity. Analysis of the test's results is based on the 'B - A value'. This value is obtained from subtracting response values of PPD-A from response values of PPD-B. To maximize diagnostic specificity or sensitivity, the B – A value should be higher than a specific adjustable threshold value.

The assay has scientific value but is not used for routine diagnosis because it has a long incubation period, and the test must be performed immediately after the collection of the blood sample. It is quite expensive requiring difficult logistics and laboratory procedures, and various laboratories are yet to compare their results for this test, so it lacks proper credibility. (Lilenbaum and Fonseca, 2006; OIE, 2009).

1.12.4.4 Gamma-interferon assay

The Gamma-interferon Assay is used as an alternative test to the tuberculin test for international trade or to identify cattle with bTB that have been missed by tuberculin testing. In this test, a whole blood sample is incubated for 16-24 hours with mycobacterial antigens such as PPD of tuberculin. This induces leucocytes in samples from cattle with bTB to release the lymphokine gamma interferon (IFN-y) which is then detected by means of a sandwich ELISA that uses two unique anti-interferon monoclonal antibodies (Coad et al., 2008; Wood et al., 1990). Comparison of the gamma interferon response obtained using bovine and avian PPD is routinely used to optimise the specificity of the test (Coad et al., 2008; Whelan et al., 2004).

Blood samples are collected and quickly taken to the laboratory and the test is conducted within a 24-hour period (Coad et al., 2007; Ryan et al., 2000). The gamma interferon assay detects more early infections than the skin test especially when conducted in parallel with the ELISA (Gormley et al., 2006).

The Gamma-interferon Assay has often been used in Australia and South Africa for testing the African buffalo. Within 7 to 8 hours the blood samples collected must be taken to the laboratory. This is a major challenge. Also, the equipment required for the test is expensive and requires trained technicians. However, this test has the advantage of requiring just a single visit, reducing cost of transportation to the farm. A person with minimal training can collect blood samples from the herd and only one blood test needs to be carried out hence the animal is captured only once (Cyrithia, 2003; Wood and Jones, 2001).

1.12.4.5 Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) is the most common test in the detection of antibodies and antigen to bTB. This is basically due to its high levels of sensitivity and specificity. Even though several studies have illustrated that the ELISA for bTB diagnosis does not have higher specificity than other methods of diagnosis, it is used as a complementary and not an alternative test (Azra and Yasmeen, 2001). The irregularity and delay of the humoral immune response in cattle to infection limits the sensitivity of the test. Its specificity is low on complex antigens like culture filtrates or tuberculin. It is however possible to increase the specificity of ELISA by simply using a combination of different antigens including proteins such as MPB 70 (antigen secreted by *M. bovis*) and MPB 83 (cell wall lipoprotein). The immune responses to both MPB70 and MPB83 gives an antigen-induced cell mediated response that is strong enough in detecting early stage of TB infection and TB infection in anergic cattle (Lilenbaum et al., 2011). The advantage of the ELISA method is its simplicity,

low cost, and it can easily be automated to process large numbers of samples (Boadella et al., 2011; Lilenbaum et al., 2011; Ramos et al., 2014).

1.12.5 Molecular diagnostic techniques

1.12.5.1 Polymerase chain reaction

One technique that is widely recognised and used for diagnosis and molecular biological research is the polymerase chain reaction (PCR). It is a ubiquitous technique, and it involves the in-vitro amplification of specific nucleic acid (NA) sequences by means of DNA polymerase enzyme (Mullis, 1990; Persing et al., 1993). It uses oligonucleotide primers in the amplification of targeted sequences of NA and involves several rounds of denaturation, primer annealing and primer extension by the enzyme DNA polymerase which is thermostable (David et al., 2019; Garibyan and Avashia, 2013).

In this technique, the chosen primers used determine the specificity of the amplification process, enabling millions of specific copies of DNA sequences to be made. These DNA sequences can be a part of a gene or a stretch of nucleotides (David et al., 2019). The PCR process requires the specific DNA sequence of interest to be heated, which denatures the double-stranded DNA and the specific synthetic oligonucleotide primers bind to the complementary target DNA sequence. The heat stable DNA polymerase enzyme extends from the primers, creating new a strand of complementary DNA. Repeating this process about 25 to 40 times creates millions of copies of DNA sequence.

Detection of the amplified sequence can be done under ultraviolet light by gel electrophoresis after staining with ethidium bromide or by hybridisation to radioactive or nonradioactive probes after transfer onto synthetic membranes (David et al., 2019). The PCR technique is highly sensitive and specific. There has been improvement to the PCR technique since it was first introduced, which includes criteria of primer selection, detection methods of amplified

products, contamination control, design of equipment and automation of the assay system (David et al., 2019; Sritharan and Sritharan, 2000).

The investigation of TB in humans and cattle has been transformed by molecular approaches to diagnosis using PCR (Ahmed et al., 1998; Cedeño et al., 2005; Sritharan and Sritharan, 2000; Vitale et al., 1998). Some of the advantages of using PCR in diagnosing pathogens include the short identification time and the improved level of DNA sequence detection in clinical specimens (Clarridge et al., 1993).

The PCR can rapidly detect different target sequences including the *IS6110* insertion sequence (Brisson-Noël et al., 1989; Eisenach et al., 1991; Hermans et al., 1990; Pao et al., 1990; Thierry et al., 1990). The *IS6110* insertion sequence has only been detected in member species of the MTC, in multiples copies. The typical *M. tuberculosis* variants found in humans often have 8 to 20 copies of IS*6110* element, while 2 to 6 copies are found in *M. bovis* strains (Collins et al., 1993; Cousins et al., 1993b; van Soolingen et al., 1994; van Soolingen et al., 1991; van Soolingen et al., 1992). *M. bovis* BCG has just a single copy of IS*6110* (Hermans et al., 1991; Szewzyk et al., 1995).

1.12.5.2 Real-time polymerase chain reaction

Real-time polymerase chain reaction (Real-time PCR), also called quantitative PCR (*q* PCR) is a laboratory-based technique that allows for both detection and quantification of the PCR product in a sample. It allows one to monitor the progress of the reaction during the exponential amplification phase of the reactions as it occurs in real time, which allows highly accurate quantification of the amount of starting material in the samples (Higuchi et al., 1993; Maddocks and Jenkins, 2017; VanGuilder et al., 2008). The three major steps that make up each cycle in a real-time PCR reaction are denaturation, annealing and extension. Reactions are generally run for 40 cycles (Maddocks and Jenkins, 2017). In real-time PCR, the amount of DNA is measured after each cycle via fluorescent dyes. Fluorescent reporters commonly used in real-

time PCR include the double-stranded DNA-binding dyes, or dye molecules attached to sequence-specific probes that hybridize with PCR products during amplification (Löfström et al., 2015). Fluorescent signal can be seen increasing in proportion to the number of amplicon or molecules produced as the reaction progresses. This is measured by the aid of an instrument that combines thermal cycling with scanning for fluorescent signals (Löfström et al., 2015). The instrument can also generate an amplification plot which is created when there appears significant fluorescence from each sample which is plotted against cycle number, and this represents the accumulation of product over the duration of the entire reaction process. This value is called cycle threshold (C_t) or cycle quantification (C_q) (Babafemi et al., 2017; Fairfax and Salimnia, 2010; Löfström et al., 2015; Roy et al., 2019).

Real-time PCR technique can increase detection efficiency of TB disease in animals and human in low bacillus load samples. It requires approximately 6 copies/ml of TB DNA in comparison to smear microscopy that requires 5000–10,000 bacilli/ml (Rachow et al., 2011). This molecular technique has been shown to detect TB with higher sensitivity and specificity directly from positive cultures or clinical specimens within 2 hours. Real-time PCR has been used in several studies to detect TB in animals and humans (Babafemi et al., 2017; Bainomugisa et al., 2015; Barletta et al., 2014; Reddington et al., 2011).

1.12.5.3 Restriction fragment length polymorphism

Restriction fragment length polymorphism (RFLP) is a nucleic acid base technology which has a high discriminative power and reproducibility and because of this, it has been considered as the gold standard for genotyping *M. tuberculosis* internationally. Studies on TB epidemics have used this method, and it has been used in epidemiology as a distinguishing tool for endogenous re-infection and reactivation (Guomiao et al., 2006). RFLP based on the presence of the insertion sequence 1S6110 is used for typing MTC (Otal et al., 1991; Thoen and Williams, 1994). Strains of MTC have specific differences. This technology uses these differences as well

as the rate of sequences of DNA on chromosomal DNA to differentiate strains. Genomic DNA is digested with specific restriction enzymes and patterns are produced after DNA fragments are analysed on agarose gel (Patel et al., 1996). An advantage of the RFLP is that a hybridization stage with probes is not necessary. On the contrary, it produces a complex pattern from many fragments whereas only a few RFLP types are observed. This makes interpreting results from this analysis cumbersome. RFLP technology is technically demanding, it requires large amount of DNA and hence restricted to use with cultures of mycobacteria, which take a long time to grow (about 20-40 days), making the process slow. It is also expensive and requires sophisticated software to analyse the result (Tewodros and Girja, 2012; Thoen and Williams, 1994).

1.12.5.4 Variable numbers of tandem repeats

Variable numbers of tandem repeats (VNTR) is a location in DNA where a short sequence of nucleotides (20–100 bp) is organized as a tandem repeat (Ellenbroek and Youn, 2016). In VNTR typing, the number of tandems repeats is assessed by PCR amplification with primers targeting the flanking regions of each repeat locus.

VNTR typing is a relatively simple technique, however it is not well standardized and large databases are missing (Harris, 2006; Hilty et al., 2005). Therefore, VNTR typing is often used as an additional tool to sub differentiate clusters of strains with identical spoligotype patterns (Smith et al., 2006a).

M. tuberculosis typing has been proven to be effective using the portable method of mini-satellite that bear VNTRs (Kanduma et al., 2003). The *M. tuberculosis* genome has been identified as having 25 loci called mycobacterium interspersed repetitive units (MIRUs). Laboratory demonstrations have shown that 12 loci differ in the number of tandem repeats, and in the sequence between repeat units in several of them. The PCR typing method is based on

these loci. The PCR based typing method has similar discriminatory abilities with IS6110 strains with high copy numbers. It is even better for strains with low copy numbers (Lee et al., 2002). In laboratories equipped with automated sequencers, this method can be employed for typing on a large scale (Supply et al., 2000). It is easy to set up and yields result within 24hours. The method can be reproduced and is sensitive and specific for the *M. tuberculosis* complex strains at their various stages of evolution (Kanduma et al., 2003). Due to the infrastructure requirement, this method is limited to large research or reference centres (Kanduma et al., 2003). In comparison with the RFLP and Spoligo-typing, this MIRU-VNTR typing showed more specific patterns.

1.12.5.5 Spoligotyping

Spoligotyping is also called spacer oligonucleotides typing and is a method for simultaneous detection and strain differentiation of mycobacterium. This technique is based on PCR amplification of highly polymorphic direct repeat (DR) locus (Kamerbeek et al., 1997). The DR region is amplified, and then the amplified products are differentially hybridized with oligonucleotides that complement the spacer regions between the DR regions. The number and type of spacer regions determine the spoligotype pattern used to categorize similar and different strains (van Soolingen et al., 1995). Spacer sequences, which vary from strain to strain, are recognized by a spot in a fixed position of the hybridization membrane. The sensitivity and specificity of spoligotyping was found to be 98% and 96% respectively. The advantage of this method is that it is fast, reliable and it can differentiate the various strains in all the MTC species. It can also distinguish *M. tuberculosis* from *M. bovis* and it is useful in the identification of outbreak and contact tracing of TB (Ameni et al., 2011; Kamerbeek et al., 1997).

1.13 Control and eradication of tuberculosis

To control or eradicate TB in cattle and humans properly, it is important that the infection is detected early. Infected animals should be quarantined, and infected humans should undergo treatment with antibiotics. The target population should be vaccinated as a preventive measure to attenuate the disease (Goodchild and Clifton-Hadley, 2006; Pavlik, 2006). Raw milk should be sterilized routinely to prevent transmission of *M. bovis* to humans through consumption of infected milk by a strict pasteurization procedure which involves a process in which raw milk is heat treated at specific temperature and time to kill any disease-causing pathogen that may be found in the milk (Michel et al., 2015; Wilson, 1943). International standards for bTB control are mainly focused on the Test-and-Slaughter (TST) policy where the TST is used periodically to identify infected herd and reacting cattle are removed from the herd (Abernethy et al., 2006; Good, 2005).

In high-income countries, it is mandatory to report the infection of animals with *M. bovis.* Infected herds are quarantined, and bovine tuberculin skin positive reactors are traced and re-tested. Animals that have not been tested and deemed TB free are confined, and movement of animals from a known infected herd or endemic areas are restricted (DEFRA, 2011; OIE, 2009). In low and middle-income countries, especially where the TST policy is not appropriate a similar but slightly different program, the test-and-segregation, is more useful for control (WHO, 1994b). In this program, initially infected herds are segregated and then those that react are slaughtered in phases. Nations which have proper eradication strategies often use both methods, employing the use of the test-and-segregation during early stages and then finally reverting to the TST approach (CFSPH, 2009). During meat or slaughter inspection, cases are traced back to the farms where they came from (DEFRA, 2011).

Proper education on farm management practices is necessary to reduce the load and spread of bTB generally. With the right sanitary approaches, the bacterium will be controlled within a

herd and between herds as well. Also, this will prevent humans from being exposed to the mycobacterium (Wilsmore and Taylor, 2008).

However, eradication of *M. bovis* is not easy. Complications include how some of its reservoir hosts are wild animals e.g., Eurasian badgers (*Meles meles*) in the UK, brushtail possums (*Trichosurus vulpecula*) in New Zealand. Another effective approach to reduce the incidence of transmission of the bacterium is by culling. While this may be effective, care has to be taken as it could lead to economic loss when the animals are culled (CFSPH, 2009).

1.14 Constraints to diagnosis and control of bovine tuberculosis in Africa

Bovine TB is found worldwide. However, some countries have never detected bTB, while others have rid themselves of the disease through deliberate attempts by embarking on TB prevention and control programmes. The incidence of bTB is highest in many countries in Africa, parts of Asia and the Middle East (Ayele et al., 2004; Cosivi et al., 1998a).

Globally, the tuberculin test is the gold standard for bTB control. However, it poses a challenge in Africa. The challenges include the need for trained personnel, funds to carry out the testing, lack of individual animal identification and the farms are often distant with bad access roads, thus making it more challenging for the personnel to return in the specified 72 hours to carry out the inspection of cattle. Also, most cattle are being moved in search of water or grazing land, or to avoid harmful insects, hostile weather, or harmful social environment and hence they might not be found to be in the same place when the professional returns for inspection (Michel et al., 2004). Control is also difficult because unlike in developed countries where slaughter is almost always carried out at abattoirs, in many African countries there are few abattoirs, and most slaughters are carried out informally by individuals. This does not allow for proper ante and post-mortem inspection of cattle (Michel et al., 2004).

Bovine TB control also faces major setbacks in developing countries due to limited or lack of necessary diagnostic equipment. The direct smear microscopic observation method often employed in these countries is grossly insufficient to identify and differentiate the specific mycobacterium causing the infections (Shitaye et al., 2007).

1.15 The study area

The study was conducted in Plateau State because of the availability of data and resources. Plateau State is in the north central geopolitical zone of Nigeria between latitude 9.2422°N - 10.1153°N and longitude 8.6957°E - 9.5210°E, covering an area of 26, 899 km^2 with an altitude range from around 1,200 meters to a peak of 1,829 metres above sea level (Figure 1.7). It lies within the Guinea savannah vegetation zone of Nigeria with an average annual rainfall of 1000 -1500 mm. The state has a tropical climate with near temperate climate in the northern part of the state. Its average temperature is between 13-22^{0}C. The climate and rocky terrain are very conducive for livestock and arable crop production. It was historically known to be free from trypanosomiasis and tsetse flies, which encourage livestock production in much of the region, and this, plus abundance of pasture, has attracted pastoralists and their cattle to the area. However, since 1982 there have been reports of African animal trypanosomiasis and tsetse flies in Plateau State. The state plays a significant role in the cattle industry nationally. It has an estimated human population of 3.5 million people of which about 300,000 are Fulani Pastoralists known for their cattle breeding and pastoral activities, and it accommodates approximately 7% of the national cattle herd, estimated to be 1.07 million, which are mainly reared by the Fulani pastoralists. While the state is best known for its mining activity, the major occupation is farming (arable and mixed farming) (Government of Plateau State, 2019; Majekodunmi et al., 2013; Wikipedia contributors, 2020).

Plateau State is known as “The Home of Peace and Tourism” in Nigeria and it is divided into 17 Local Government Areas, namely Barkin Ladi, Bassa, Bokkos, Jos East, Jos North, Jos

South, Kanam, Kanke, Langtang North, Langtang South, Mangu, Mikang, Pankshin, Qua'an-pan, Riyom, Shendam and Wase.

The capital of Plateau State is Jos. It is the administrative capital and largest city in Plateau State. It is made up of Jos East, Jos South and Jos North Local Government Areas and it is popularly called "J-Town". It lies between latitude 9°56' North and longitude 8°53' East, having a total land area of 8,600km2. It has a population of about 900,000 residents based on the 2006 census (Wikipedia contributors, 2020).

Figure 1. 7 Map of Nigeria, Showing the position of Plateau State

Source : https://guardian.ng/wp-content/uploads/2015/03/Plateau-state-map.jpg

1.16 Breed of cattle in Plateau State, Nigeria.

The popular indigenous breeds of cattle found in Nigeria are Adamawa Gudali, Azawak, Keteku, Kuri, Ndama, Muturu, Red Bororo, Sokoto Gudali, Wadara, and White Fulani (Babayemi et al., 2014; Pagot, 1992). The White Fulani breed is the most numerous and widely distributed in Nigeria and is estimated to represent 37% of the national herd (Alphonsus, 2012; Blench, 1999; Meghen et al., 1999). The most common breed of cattle found in Plateau State are the Muturu, Red Bororo and White Fulani (Figures 1.8, 1.9 and 1.10 respectively).

1.16.1 Muturu

The West African dwarf short-horn or Muturu is small bodied, and blocky in conformation with black or black and white patches of coat colour (Figure 1.8). The adult weight ranges from 109-204kg (Olufunmilayo, 2001). The Muturu breed on the Jos Plateau is distinctly larger than the low-land animals. The breed is trypan tolerant and tolerates ticks and tick-borne diseases. At present, the major concentrations of Muturu are in the South-East of Nigeria, in the Cross River area and among the Tiv people in and around Makurdi, capital of Benue State (Meghen et al., 1999). According to Adang et al. (2015), the Muturu breed has been known to have cultural values and is used for socio-cultural purposes. It is sacred and dedicated to shrines.

Figure 1. 8 Photograph of Muturu breed of cattle. 2017

1.16.2 Red Bororo

Also called Red Fulani, Mbororo, Wodabe, Fellata, Abori, Bodadi, Brahaza and Rahaji. The Red Bororo has a reddish-brown coat, pendulous ears, and thick lyre-shaped horns (Figure 1.9). The adult weight ranges from 400-500kg for males and 350-450kg for females. It is the third most common breed in Nigeria. It is used for meat and milk although they have a low milk yield. This breed is mostly found in Eastern Mali, Niger, Northern Nigeria, Weston Chad, Northern Cameroon, Central African Republic, Western Sudan and Gambela region of Western Ethiopia. The Red Bororo has a Zebu hump on its back that contains fatty deposits which stores energy and water and hence it can travel without food or water for a considerable length of time. It has a nervous and fierce temperament and is sometimes difficult to handle, with the bulls being especially difficult (Blench, 1993; Meghen et al., 1999).

Figure 1. 9 Photograph of Red Bororo breed of cattle. 2017

1.16.3 White Fulani

Also called Bunaji. The White Fulani is the most numerous and widespread breed of cattle in Nigeria (Blench, 1993; Meghen et al., 1999). It is mostly reared by the Fulani pastoralists, with a white coat, long lyre shaped horns with a distinctive hump; its height is about 130 cm (Figure 1.10). A bull weighs about 500 kg and a cow 325 kg. The breed is well adapted to long distance travel due to the shallowness of the body and its lack of width which gives the breed a leggy

appearance. The White Fulani cattle are heat tolerant and sluggish. They mature late, have long intervals between calvings and short lactation length. They are used for meat and milk. They provide much of the beef consumed throughout Nigeria (Alphonsus, 2012; Capitaine, 1972).

Figure 1. 10 Photograph of White Fulani breed of cattle. 2017

1.17 Justification for the study

TB remains an important public health disease, and is a significant zoonosis transmitted from cattle to humans and vice versa. Many studies have shown that there are many factors responsible for the spread and persistence of TB in developing countries like Nigeria such as demographic factors, eating habits, poverty, illiteracy, culture, the burden of HIV/AIDS, and close proximity with animals. However, there are several difficulties in the control and diagnosis of bovine and human TB in Nigeria which include:

1. Paucity of information on both human TB of zoonotic origin and bTB in Nigeria and hence the prevalence of the disease and its public health importance is not known. There is also general underestimation of the importance of zoonotic TB by the public health sectors in Nigeria
2. Control policies for bTB are inadequately implemented in Nigeria. Control measures such as abattoir meat inspection and disease surveillance are mandatory according to laws in Nigeria but have not been enforced and are poorly carried out or not done at all

3. There is lack of standardization of meat inspection protocols in abattoirs in Nigeria
4. The use of molecular diagnostic methods for species and strain identification has not been fully exploited in Nigeria; hence there is no data on the mycobacteria species responsible for causing the disease
5. In TB health centres or hospitals in Nigeria, the diagnoses of TB are limited to direct smear microscopy in which the organism needs several weeks to grow. So, the proportion of zoonotic TB compared with human transmitted TB is not known

1.18 Aims and objectives of the study

1.18.1 Overall aims of the study

The aim of this thesis is to assess the epidemiology of TB in cattle and human, risk factors for TB prevalence, level of knowledge on bTB and the zoonotic preventive practices demonstrated by livestock workers in Plateau State, leading to an enhanced implementation of the TB control program in cattle and humans with the ultimate aim of ending the TB epidemic in Nigeria.

1.18.2 Objectives

1. To determine the prevalence and demographic factors associated with the risk of detecting suspected TB lesions from 2001 to 2016 using retrospective records of routine post-mortem examination of slaughtered cattle from the Jos abattoir, Plateau State (chapter two)
2. To determine the prevalence and demographic factors associated with TB in humans from 2001 to 2015 using retrospective laboratory records on TB from the Jos University Teaching hospital, Plateau State (chapter two)
3. To determine the prevalence of suspected TB lesions in slaughtered cattle based on data obtained from detailed post-mortem examination of carcasses at the Jos abattoir, Plateau State (chapter three)

4. To isolate and characterize mycobacterial agent from suspected TB lesions in cattle slaughtered at the Jos abattoir, Plateau State using Real-time Polymerase Chain reaction (chapter three)
5. To assess the level of knowledge on TB and the zoonotic TB preventive practices and associated determinants by interviewing abattoir workers and Fulani pastoralist in Jos, Plateau State (chapter four)

CHAPTER TWO: PREVALENCE AND RISK FACTORS FOR TUBERCULOSIS IN CATTLE AND HUMANS (A RETROSPECTIVE OBSERVATIONAL STUDY)

2.1 Introduction

Nigeria is the most populous country in Africa with a population of over 200 million people, an estimated cattle population of 20.5 million cattle and an unknown population of wildlife (Krauss et al., 2004; World Bank, 2019). The growing population density, the close relationship of animals and humans, the consumption of infected meat and unpasteurized milk and traditional livestock husbandry practices, where humans co-habit with livestock, increase the likelihood of the zoonotic and reverse zoonotic TB, with a potential impact on animal health, animal productivity, animal international trade and human health (Ayele et al., 2004; Cadmus et al., 2019). This represents a threat to human livelihoods by compromising sustainable food supply, income and social status (Krajewska et al., 2017; Torgerson and Torgerson, 2010; WHO, 2006).

The presence of TB in cattle is well established in Nigeria based on macroscopic lesions at meat inspection (Abubakar et al., 2011a; Adang et al., 2015; Agbalaya et al., 2020; Ibrahim et al., 2010). In many developed countries, diagnosis of TB in cattle is done by tuberculin testing, culture, and molecular diagnostic techniques (Humblet et al., 2009; Schiller et al., 2011). However, in Nigeria and other developing countries this is not possible due to lack of equipped laboratories and trained personnel as well as high cost of running these tests. Another constraint is the cattle management system which is predominately done by the Fulani pastoralists who are constantly moving their herds in search of food and water (Cadmus, 2019).

As in many resource-poor countries, the primary laboratory tool used for monitoring TB in humans in Nigeria is the microscopic examination of stained sputum smear (Parsons et al., 2011), while the routine abattoir post-mortem inspection of carcasses is the only strategy applied to monitor diseases such as TB in animals in Nigeria. Although results from these

routine inspections may be prone to error and subjectivity by the inspector, they nevertheless provide useful information on the prevalence of diseases including TB among others (Awah Ndukum et al., 2010). It is also important in the protection of public health with regard to consumer protection and control of zoonotic diseases like TB (Awah Ndukum et al., 2010; Igbokwe et al., 2001).

Disease control is largely absent in cattle in Nigeria. Consequently, the majority of the human population is at risk of exposure to bTB, and globally, the occurrence of zoonotic TB likely mirrors the TB prevalence in cattle (Müller et al., 2013). With the high TB prevalence in humans and the emergence of acquired immunodeficiency syndrome, there is a need to investigate the importance of bTB and how it contributes to the overall burden of TB infection, especially in developing countries (WHO, 2014).

Data on human and animal tuberculosis in Plateau State, Nigeria are collected by the State Ministry of Health and Ministry of Agriculture, respectively. Unfortunately, these data are left unpublished, the public is not aware of the presence of the disease and therefore continue to engage in activities that may increase their risk of acquiring tuberculosis. The present study was conducted to contribute to the knowledge about the epidemiology of TB in humans and cattle in Nigeria. Findings from this work can inform intervention strategies for the control of TB and for future TB monitoring.

2.2 Aims of the study

The study aim was to investigate TB prevalence and factors associated with TB in cattle presented for slaughter at Jos Abattoir and humans tested for TB at Jos University Teaching Hospital (JUTH) using retrospective data.

2.3 Study site

At the 2006 census, Plateau State had a human population of 3,206,531, comprising of 1,598,998 males and 1,607,533 females ("Report of Nigeria's National Population Commission on the 2006 Census," 2007). The State is divided into three geopolitical zones: Plateau North, Plateau Central and Plateau South (Figure 2.1). The only registered abattoir in Plateau State is in Jos, the administrative capital and largest city of Plateau State within Jos South Local Government Area (LGA) of Plateau State (Okeke et al., 2016). The Jos University Teaching Hospital, also located in Jos, is the major TB treatment centre in Plateau State and serves as a referral centre for health facilities in the state.

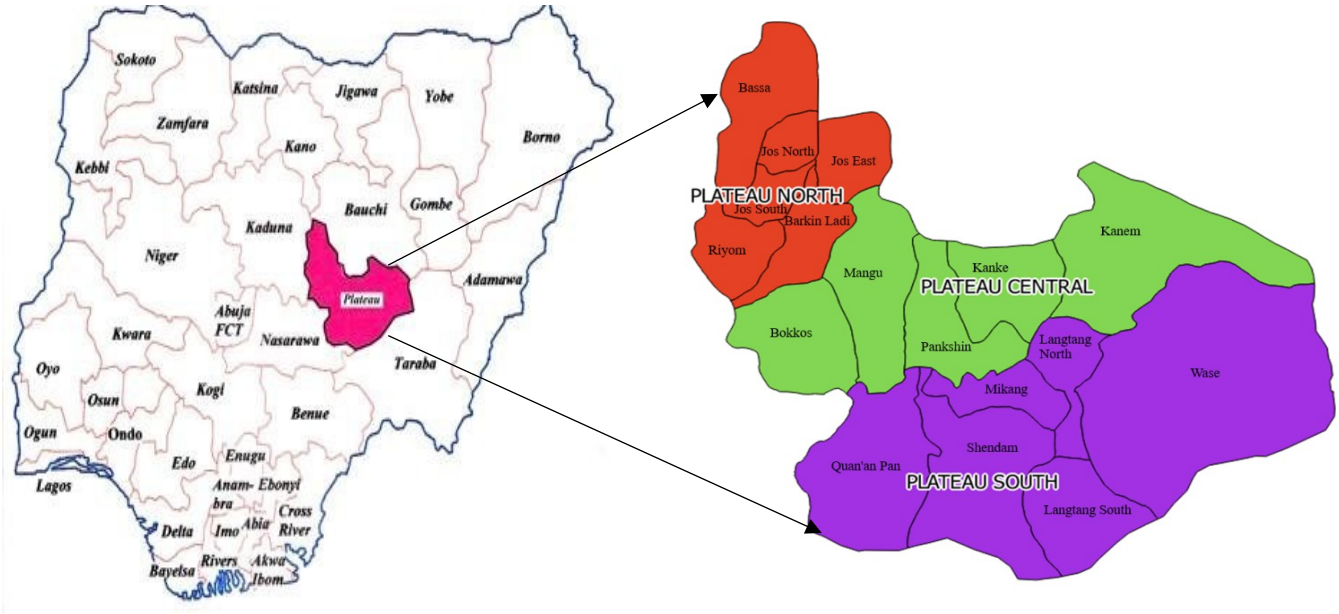

Figure 2. 1 Map of Plateau State in Nigeria showing the three geopolitical zones of the state (Plateau North, Plateau Central and Plateau South) with the 17 Local Government Areas of the state

Source: Ifende et al. (2019) and https://umar-yusuf.blogspot.com/2017/10/map-of-nigeria-senatorial-districts-by.html

2.4 Methods

2.4.1 Study design and data collection

A retrospective observational study utilised secondary data comprising routine meat inspection monthly records of suspected TB in cattle slaughtered at the Jos Abattoir from 2001 to 2016. The data elements collected included TB status (presence or absence of lesions suggestive of TB detected at post-mortem), age group, gender, breed, and location (geopolitical zone of settlement).

Similarly, a retrospective observational study of laboratory records on TB in humans (including both human TB and bTB) at JUTH was retrieved for a period of 15 years from 1st January 2001 to 31st December 2015. Data elements collected included TB status based on laboratory diagnosis, age group, gender, and location (geopolitical zone of origin).

Both cattle and human data were paper records and I collected them manually according to month and year. The cattle data was kindly provided by the Jos abattoir with permission from the Plateau State Ministry of Agriculture and Rural Development and the human data was provided by the Infectious Disease Department of JUTH.

2.4.2 Study populations

2.4.2.1 Bovine study population

The bovine study population comprised all cattle slaughtered at the Jos Abattoir from 1st January 2001 to 31st December 2016 meeting the study criteria (section 2.4.5).

A total of 179,362 were slaughtered during this period of which 163,640 met the study criteria (section 2.4.5).

2.4.2.2 Human study population

The human study population comprised all patients tested for TB at the JUTH meeting the study criteria (section 2.4.5). A total of 47,173 patients met the study criteria (section 2.4.5).

2.4.3 Outcome variable – TB status

Diagnosis of TB in cattle was based on routine post-mortem examination of carcasses to detect cattle with lesions suggestive of TB (Herenda et al., 2000). Cattle with lesions suggestive of TB was referred to as TB cases and those without lesions suggestive of TB were referred as non-TB cases.

Diagnosis of TB in patients was based on ZN sputum smear microscopy to detect AFB. A TB case in humans is when AFB was detected in the sputum and a non-TB case is when no AFB was detected in the sputum.

2.4.4 Explanatory variables studied as potential risk factors for TB

2.4.4.1 Age

Age refers to the years of life at the time when cattle was slaughtered in the abattoir or at the time of a ZN sputum smear microscopy test to detect AFB in humans. Ages were grouped and analysed as a categorical variable. Ages of cattle were categorised into three age groups: <5 years, 5-10 years and >10 years. Ages of humans were categorised into four age groups: <20 years, 21-40 years, 41-60 years and > 60 years.

2.4.4.2 Gender

Gender of humans and cattle was recorded as a dichotomous categorical variable and categorised as male or female.

2.4.4.3 Season

Season was categorised into wet and dry season. In plateau State, the wet seasons extends from the month of May to October and the dry seasons from November to April (Lee, 1972).

2.4.4.4 Location

Location refers to the geopolitical zone in Plateau State from where human patients were referred or the settlement area of origin of slaughtered cattle. Location was grouped into three categories: northern geopolitical zone, central geopolitical zone, and southern geopolitical zone.

2.4.4.5 Breed

Breed of cattle refers to groups of cattle having homogenous appearance, behaviour, and/or other characteristic that distinguish it from other cattle of the same species. Breed was recorded as a categorical variable and the categories were White Fulani, Red Bororo, Muturu and mixed breed.

2.4.5 Study inclusion and exclusion criteria

Exclusion criteria:

- Animals with incomplete data or missing information on age, gender, breed, and location. There were 15,722 animals excluded from this study. Out of the animals excluded, data elements that were not filled out in the record includes, age for 10,702 animals, gender for 4,022 animals, breed for 422 animals and location for 576 animals.
- Hospital patients with incomplete data or missing information on age, gender, and location. There was no missing information hence no patient was excluded.
- Patients who presented to the hospital with other disease conditions but were also tested for TB.

Inclusion criteria:

- All animals with complete data on explanatory variables were included in the study. A total of 163,640 animals were included.
- All patients presented to the hospital with signs and symptoms of TB and that were tested for TB were included in the study. A total sum of 47,173 patient was included in this study.

Based on these exclusion and inclusion criteria, the degree of completeness of the data reports on cattle slaughter at the abattoir was calculated to be 91.23% and for human data reports the degree of completeness was 100%.

Human data reports on TB for 2016 was not available at the time of this study, hence only data from 2001-2015 was included in this study.

2.4.5 Statistical analysis

2.4.5.1 Data collation, checking and storage

Data obtained were stored in an Excel spreadsheet 2017 (version 1.0) and checked for correctness by computing frequencies (pivot tables) using Stata/MP version16.0 statistical software.

2.4.5.2 Calculation of TB prevalence

With the use of Stata/MP version16.0 statistical software, prevalence of TB in the cattle study population (as defined above 2.4.2.1) was calculated for each category as the proportion of cattle slaughtered at the Jos Abattoir with lesions suggestive of TB from 2001 to 2016 and expressed as a percentage: -

$$\text{TB prevalence in slaughtered cattle} = \frac{\text{Number of cattle with lesions suggestive of TB}}{\text{Total number of cattle slaughtered}} \times 100$$

Prevalence of TB in the human study population (as defined above 2.4.2.2) was calculated as the proportion of humans tested for TB that were positive from 2001 to 2015 at the JUTH and expressed as a percentage:

$$\text{TB prevalence in humans tested for TB} = \frac{\text{Number of people that tested positive for TB}}{\text{Total number of humans tested for TB}} \times 100$$

The 95% confidence interval for proportions was calculated using the formula:

$$\hat{P} \pm z\sqrt{\frac{\hat{P}(1-\hat{P})}{n}}$$

Where $\hat{P}$ is the sample proportion, n is the sample size, and z is the appropriate value from the standard normal distribution for the desired confidence level. For a 95% confidence interval, z = 1.96

2.4.5.3 Chi-squared test

Chi-squared (χ^2) tests were used to compare numbers of TB cases of cattle and humans categorised by year, month, season, gender, age group, location, and breed (for cattle); $P<0.05$ was considered significant. SPSS IBM version 24 software was used for these Chi-squared (χ^2) tests.

2.4.5.4 Univariable risk factor analysis

A logistic regression analysis was carried out on cattle dataset (explanatory variables: year, month, season, gender, age group, location, breed) and human dataset (explanatory variables: year, month, season, gender, age group, location) to evaluate associations between each explanatory variable as a potential risk factor and the outcome (having TB or not having TB). The category with the largest number of cohorts was chosen as reference level for comparison except for 'year' in which the most current year was chosen, and 'month' in which the first month of the year (January) was chosen. For age, the youngest age group category was chosen

as the reference level. The results of this analysis were summarized using odds ratios (OR) along with 95% CIs and the associated p-values. Variables that were significant at P = <0.05 in the univariable logistic regression were subsequently included in the multivariable logistic regression model (below, 2.4.5.5). Data analysis was conducted using Stata/MP version16.0 statistical software.

2.4.5.5 Multivariable risk factor analysis

Akaike's Information Criterion (AIC) was used in combination with the inclusion criteria for explanatory variables ($p < 0.05$ in the univariable analysis) to assist in assessing different possible models and determine which one is the best fit model for the final multivariable logistic regression analyses. Risk factors were tested and those included with the best fitting form according to AIC in a stepwise bi-directional (forwards-adding and backwards-removing) process were included in the final multivariable model. "Season" was not included in the multivariable analysis as it co-linear with month. Based on AIC test, it was concluded that the model including "month" is a better overall fit for both the cattle and human dataset than that including season. Results of the multivariable analysis were summarized using adjusted odds ratios (AOR) along with their 95% CIs and the associated p-values. Stata/MP version16.0 statistical software was used for data analysis. $P \leq 0.05$ was considered significant.

2.4.5.6 Model checking

Model diagnostics were carried out using the Hosmer-Lemeshow test to determine the goodness of fit of the final logistic regression model. Variables rejected at either the univariable or multivariable model-building stages were tested for confounding. Variables were considered to be confounders if their inclusion changed the significance of any other variable, or if the odds ratio of another variable changed by more than 20% (Dohoo et al., 1997). Second-order interactions terms between biologically plausible pairs of variables were assessed using AIC

as part of the multivariable model optimisation. Any significant interactions terms were retained in the final model. Post-hoc power calculations showed that the model had 80% power to detect, with 95% confidence, effect sizes of at least odds ratio 1.08 for the categorical variables.

2.4.5.7 Seasonal-trend decomposition

A seasonal-trend decomposition procedure based on loess (locally weighted regression) (Cleveland and Devlin, 1988; Cleveland et al., 1990) was used to characterise the profile of bovine and human TB infections over time. Prevalence of TB were calculated for each of the two host species study populations as defined, for each month and year of the study. A Box-Cox transformation (Box and Cox, 1964) was used to normalise the prevalence data prior to seasonal-trend decomposition (Hyndman and Athanasopoulos, 2018), with the lambda parameter of the Box-Cox transformation optimised to minimise the Fisher-Pearson coefficient of skewness of the data. Following the decomposition, the autocorrelation and partial autocorrelation of the residual were plotted as correlograms. These diagrams demonstrate whether the autocorrelation function of the data displayed exceeds the expectation of random 'white noise' if one or more large spikes, or substantially more than 5% of spikes, fall outside limits of $\pm 2/\sqrt{T}$, where T is the length of the time series (Hyndman and Athanasopoulos, 2018). Graphs for these were all performed using RStudio software.

2.5 Results

2.5.1 Bovine Tuberculosis in Jos Abattoir 2001-2016

2.5.1.1 Data characteristics (cattle dataset)

The data contain records of a total of 163,640 slaughtered cattle from 2001 to 2016. The highest number of cattle slaughtered was in 2005 (n = 16,868, 10.31%), in the months of December (n = 14,219, 8.69%), in the wet seasons (n = 95,954, 58.64%) and from the central geopolitical

zone of the state (n = 97,561, 59.62%). Most of the cattle slaughtered were males (n = 112,168, 68.55%) and were less than 5 years old (n = 91,231, 55.75%). The White Fulani breed of cattle constituted over three quarter of all cattle slaughtered in the abattoir (n = 125,226, 76.53%) (Table 2.1).

Table 2. 1 Characteristic*s* of cattle dataset

		Frequency	Proportion of Total (%)
Year			
	2001	10,190	6.23
	2002	10,731	6.56
	2003	8,549	5.22
	2004	6,803	4.16
	2005	16,868	10.31
	2006	11,259	6.88
	2007	7,538	4.61
	2008	11,313	6.91
	2009	13,475	8.23
	2010	9,777	5.97
	2011	10,976	6.71
	2012	6,282	3.84
	2013	6,234	3.81
	2014	8,829	5.40
	2015	8,205	5.01
	2016	16,611	10.15
Month			
	January	12,817	7.83
	February	13,487	8.24
	March	13,606	8.31
	April	13,694	8.37
	May	13,639	8.33
	June	13,691	8.37
	July	13,607	8.32
	August	13,796	8.43
	September	13,531	8.27
	October	13,984	8.55
	November	13,569	8.29
	December	14,219	8.69
Season			
	Wet	95,954	58.64
	Dry	67,686	41.36
Gender			
	Male	112,168	68.55
	Female	51,472	31.45
Age-group			
	<5	91,231	55.75
	5 to 10	50,222	30.69
	>10	22,187	13.56
Location (geopolitical zones)			
	Central	97,561	59.62
	Northern	51,021	31.18
	Southern	15,058	9.20
Breed of cattle			
	White Fulani	125,226	76.53
	Red Bororo	28,803	17.60
	Muturu	4,019	2.46
	Mixed breed	5,592	3.42
Total		163,640	

2.5.1.2 Prevalence of TB in cattle slaughtered in Jos Abattoir

From 2001-2016, a total of 163,640 cattle that met the exclusion/inclusion criteria in section 2.4.5 were slaughtered in the abattoir, out of which lesions suggestive of TB were seen in 6,599 cattle, resulting in an overall cattle TB prevalence of 4.03% (95% CI 3.94-4.13%). The mean annual number of TB cases in cattle during the 2001 to 2016 study period was 412.4 and the corresponding median annual number was 389 cases per year.

The number of TB cases in slaughtered cattle varied highly significantly across the years of the study (χ^2=3611.5, P= <0.001). The lowest number of cases was seen in 2012 (n=24) and the highest in 2007 (n=1002). Similarly, the lowest TB prevalence in cattle (0.38%, 95%CI 0.26-0.57%) was seen in 2012 and the highest prevalence (13.29%, CI 12.55-14.08%) was seen in 2007 (Table 2.2, Figure 2.2).

The number of TB cases in slaughtered cattle also varied highly significantly across calendar months (χ^2=304.23, P= <0.001). The month of April showed the fewest TB cases (n= 428) while December showed the highest number (n= 847). The lowest TB prevalence (3.13%, CI 2.85-3.43%) was seen in the month of April and the highest prevalence (5.96%, CI 5.58-6.36%) was seen in December (Table 2.2, Figure 2.4).

The pattern of variation in monthly prevalence did not correspond particularly well to the wet (May-Oct) and dry (Nov-Apr) seasons (Figure 2.3). Although the two months with highest prevalence (Nov-Dec) were observed at the start of the dry season, prevalence fell over the remaining dry season months and by March and April TB prevalences were similar to prevalences observed during the wet season months (Table 2.2, Figure 2.4).

TB case numbers differed significantly between the two genders of slaughtered cattle (χ^2=1061.1, P= <0.001). The minimum number of TB cases was seen in female cattle (n=872) and the maximum TB case was seen in male cattle (n= 5,727). Although more than twice as

many male cattle (n = 112,168) were slaughtered than females (n = 51,472), the prevalence was significantly higher in males (5.11%, CI 4.98-5.24%) than in females (1.69%, CI 1.59-1.81%; Table 2.2, Figure 2.5).

The number of TB cases varied highly significantly among age groups of cattle (χ^2=3077.59, P <0.001). Among the age groups, slaughtered cattle less than 5 years old had the lowest number of TB cases (n=1522) and cattle within age group 5-10 years old had the highest number of TB cases (n=3,278). TB was shown to be least prevalent among cattle within age group less than 5 years old (1.67%, CI1.59-1.75%) and most prevalent in cattle within age group greater than 10 years old (8.11%, CI 7.76-8.48%) (Table 2.2, Figure 2.6).

TB cases in cattle were also shown to vary across the three geopolitical zones of Plateau State, the differences being highly significant (χ^2=668.26, P= <0.001). The least number of TB cases were among cattle from the southern zone (n=128) and the highest number of TB cases were among cattle from the central zone of the state (n= 4,800). TB was shown to be most prevalent in cattle from the central zone of the state (4.92%, CI 4.79-5.06%) and least prevalent in cattle from the southern zone of the state (0.85%, CI 0.72-1.01%) (Table 2.2, Figure 2.7).

The Muturu breed of cattle had the lowest number of TB cases, and the White Fulani breed of cattle has the highest number of TB cases (n=5070). The Muturu breed showed the lowest TB prevalence (3.48%, CI 2.96-4.10%) and the mixed breed of cattle showed the highest TB prevalence (4.40%, CI 3.89-4.97%) (Table 2.2, Figure 2.8). However, the differences in the number of TB cases across the different breeds of cattle was not significant (χ^2=5.46, P= 0.141).

Table 2. 2 Prevalence and 95% confidence intervals of TB among cattle slaughtered from 2001-2016 and Chi-squared test of association between TB cases in cattle and year, age group, gender, geopolitical zone (location), season, month, and breed

Variables		No. of cattle slaughtered	*No. of TB cases	Prevalence (%)	95% Conf. Interval (%)		Chi-squared	P-value
Year							3611.5	<0.001
	2001	10190	409	4.01	3.65	4.41		
	2002	10731	415	3.87	3.52	4.25		
	2003	8549	404	4.73	4.3p	5.20		
	2004	6803	329	4.84	4.35	5.37		
	2005	16868	611	3.62	3.35	3.92		
	2006	11259	991	8.8	8.29	9.34		
	2007	7538	1002	13.29	12.55	14.08		
	2008	11313	676	5.98	5.55	6.43		
	2009	13475	597	4.43	4.10	4.79		
	2010	9777	374	3.83	3.46	4.22		
	2011	10976	322	2.93	2.63	3.27		
	2012	6282	24	0.38	0.26	0.57		
	2013	6234	73	1.17	0.93	1.47		
	2014	8829	134	1.52	1.28	1.80		
	2015	8205	81	0.99	0.80	1.23		
	2016	16611	157	0.95	0.81	1.10		
Month							304.23	<0.001
	January	12817	550	4.29	3.95	4.66		
	February	13487	605	4.49	4.15	4.85		
	March	13606	492	3.62	3.32	3.94		
	April	13694	428	3.13	2.85	3.43		
	May	13639	506	3.71	3.41	4.04		
	June	13691	552	4.03	3.72	4.37		
	July	13607	433	3.18	2.90	3.49		
	August	13796	498	3.61	3.31	3.93		
	September	13531	448	3.31	3.02	3.63		
	October	13984	509	3.64	3.34	3.96		
	November	13569	731	5.39	5.02	5.78		
	December	14219	847	5.96	5.58	6.36		
Season							159.83	<0.001
	Dry (Nov-Apr)	67686	3225	4.76	4.61	4.93		
	Wet (May-Oct)	95954	3374	3.52	3.40	3.64		
Gender							1061.1	<0.001
	Male	112168	5727	5.11	4.98	5.24		

	Female	51472	872	1.69	1.59	1.81		
Age group							3077.59	<0.001
	<5	91231	1522	1.67	1.59	1.75		
	5 to 10	50222	3278	6.53	6.31	6.75		
	>10	22187	1799	8.11	7.76	8.48		
Location							668.26	<0.001
(geopolitical zones)	Central	97561	4800	4.92	4.79	5.06		
	Northern	51021	1671	3.28	3.12	3.43		
	Southern	15058	128	0.85	0.72	1.01		
Breed							5.46	0.141
	White Fulani	125226	5070	4.05	3.94	4.16		
	Red Bororo	28803	1143	3.97	3.75	4.20		
	Muturu	4019	140	3.48	2.96	4.10		
	Mixed breed	5592	246	4.4	3.89	4.97		
Total		**163640**	**6599**	**4.03**	**3.94**	**4.13**		

*No. with lesions suggestive of TB

P<0.05 significant level

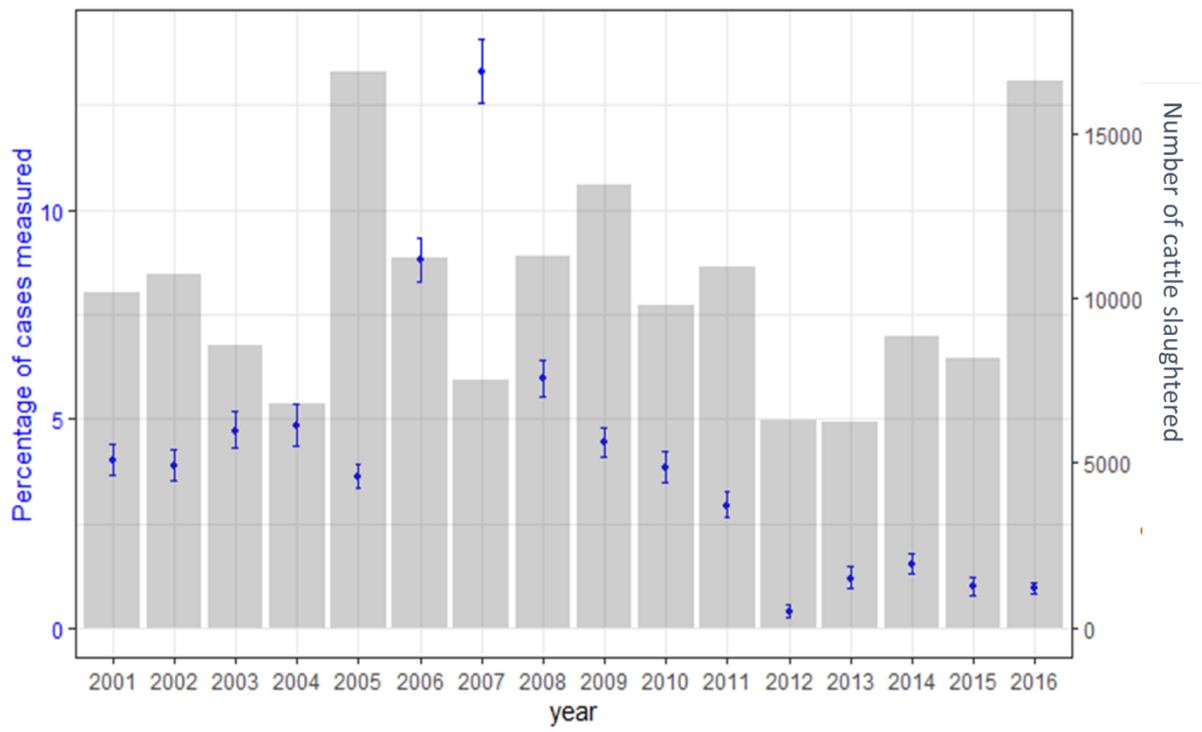

Figure 2. 2 Number of slaughtered cattle examined in each year (grey bars) and the prevalence of TB in these cattle (blue dots) with 95% confidence intervals (blue error bars)

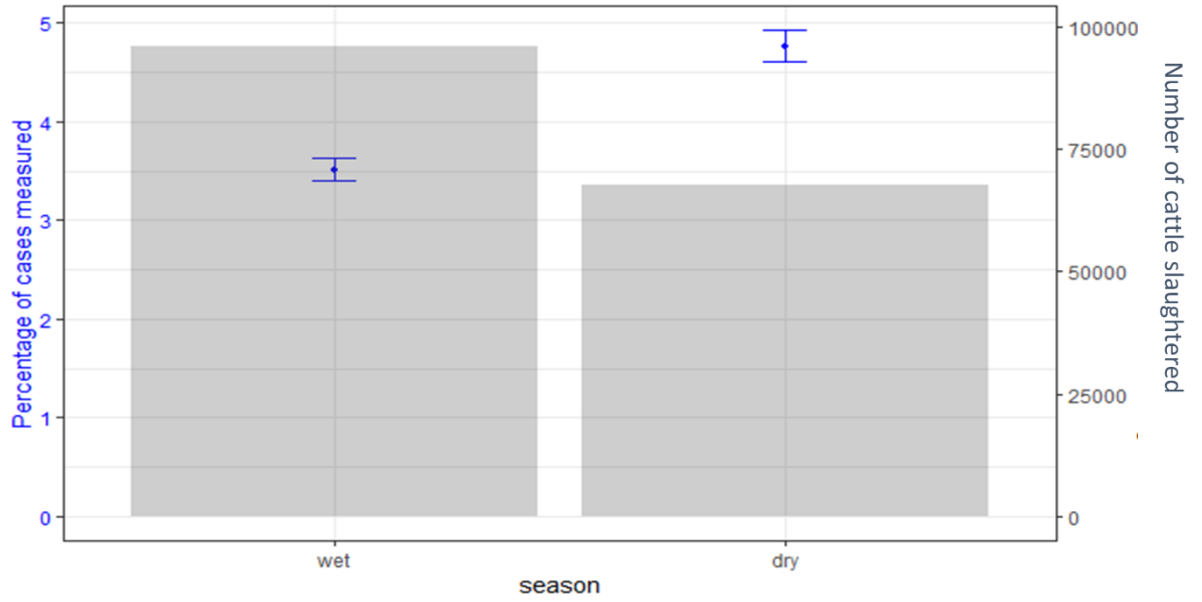

Figure 2. 3 Number of slaughtered cattle examined in each season (grey bars) and the prevalence of TB in these cattle (blue dots) with 95% confidence intervals (blue error bars)

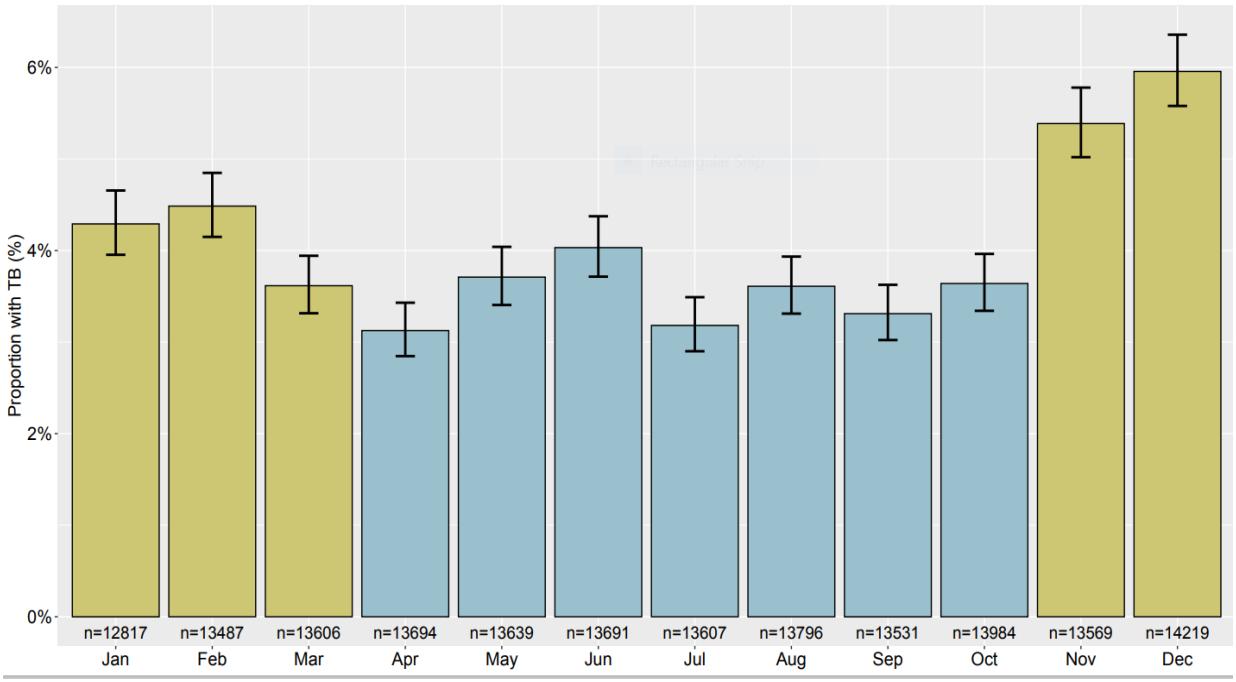

Figure 2. 4 Average monthly prevalence of TB in cattle slaughtered at Jos abattoir 2001-2016. Khaki bars: dry season; light blue bars: wet season. Error bars: 95% confidence intervals. Number of cattle examined shown below each bar

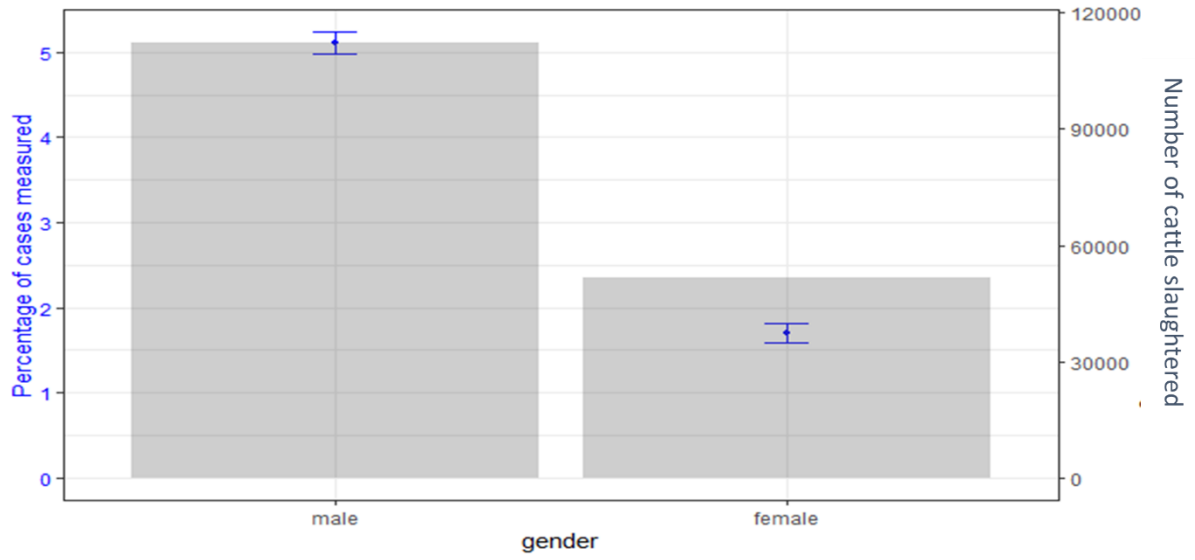

Figure 2. 5 Number of male and female slaughtered cattle examined (grey bars) and the prevalence of TB in these cattle (blue dots) with 95% confidence intervals (blue error bars)

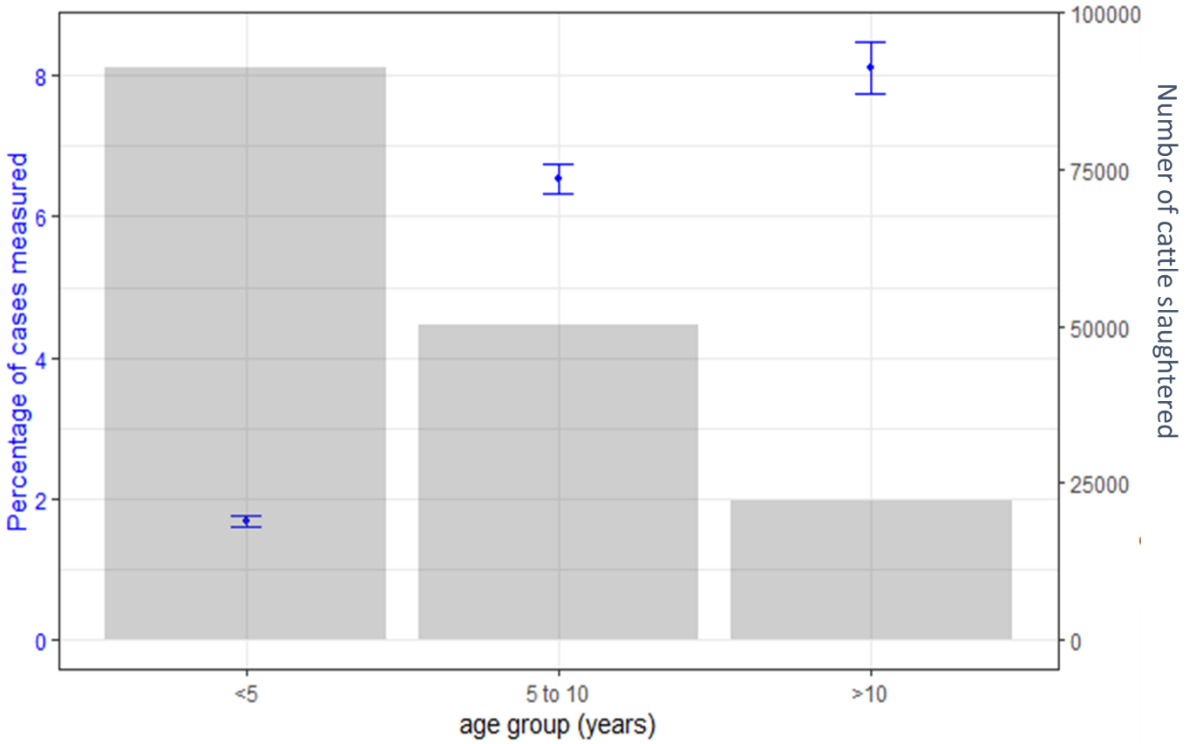

Figure 2. 6 Number of slaughtered cattle examined in each age group (grey bars) and the prevalence of TB in these cattle (blue dots) with 95% confidence intervals (blue error bars)

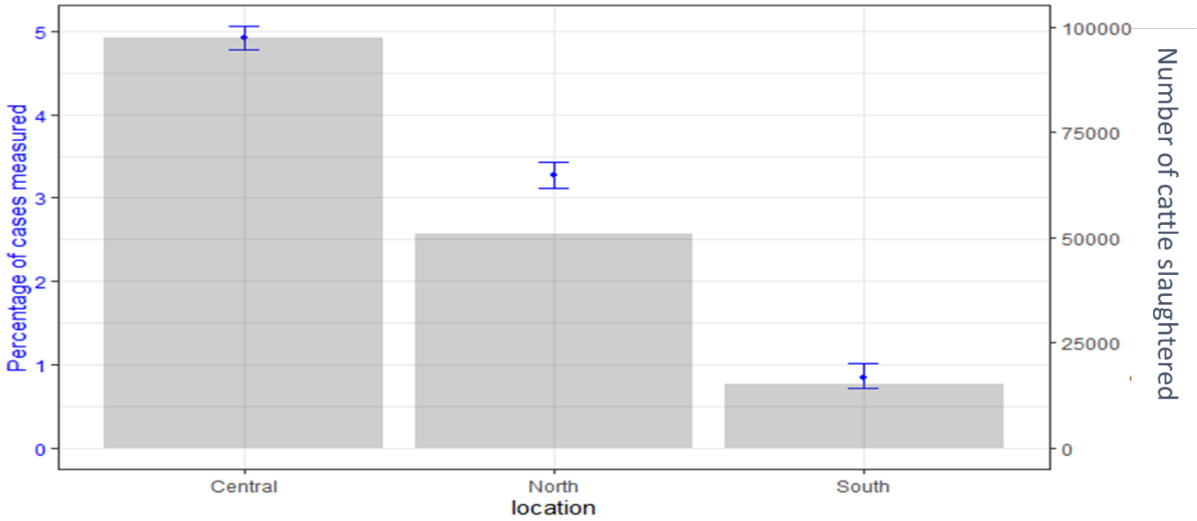

Figure 2. 7 Number of slaughtered cattle examined (grey bars) and the prevalence of these cattle (blue dots) with 95% confidence intervals (blue error bars) in each location (geopolitical zones)

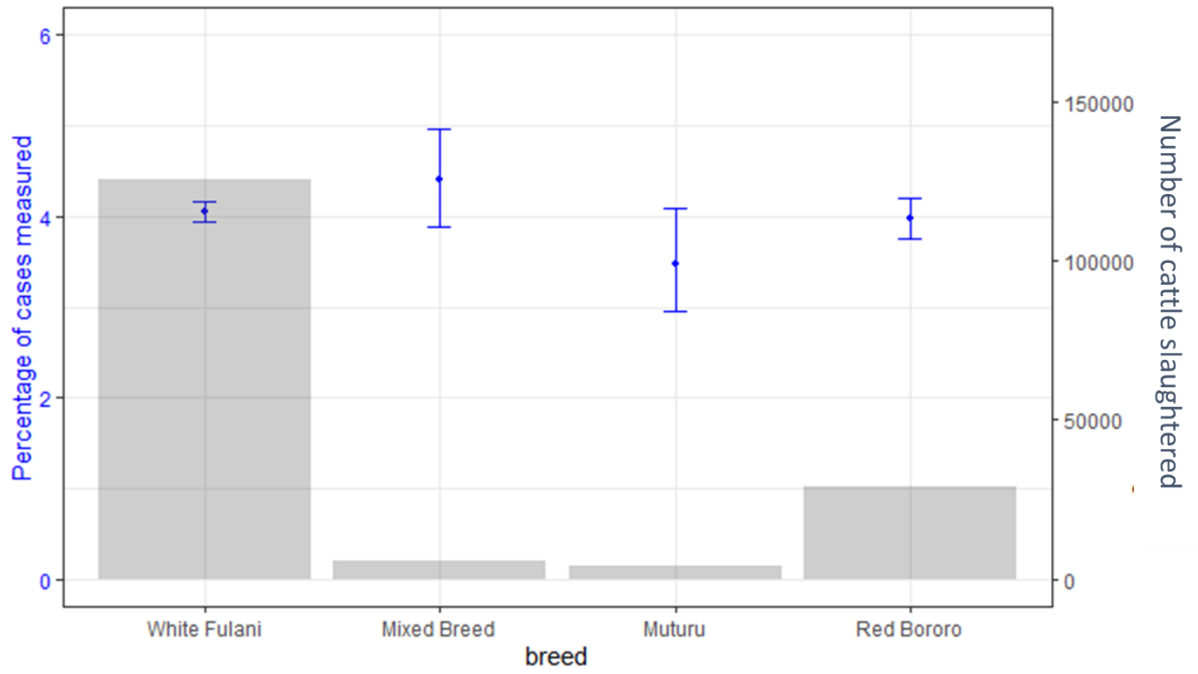

Figure 2. 8 Number of slaughtered cattle of each breed examined (grey bars) and the prevalence of TB in these cattle (blue dots) with 95% confidence intervals (blue error bars)

2.5.1.3 Univariable logistic regression analysis of risk factors for the detection of TB in cattle slaughtered at Jos Abattoir

Univariable logistic regression analysis showed that the following variables were associated with the detection of lesions suggestive of TB in slaughtered cattle: year, month, season, gender, age group, and location.

Lesions suggestive of TB were significantly more likely to be detected among cattle slaughtered in all years prior to 2013 ($p < 0.001$ for all years 2001 – 2012), compared to the reference year 2016. Over this period (2001 – 2012) the odds ratios ranged between 3.17 (CI 2.61-3.84) in 2011 and 16.07 (CI 13.55 – 19.06) in 2007. Two of these years, 2006 (OR 10.11, CI 8.53-11.99) and 2007 (OR 16.07, CI 13.55 – 19.06), showed especially high odds ratios, with confidence intervals suggesting that the prevalences in these years were significantly higher than those in all other years (highest upper 95% confidence limit for odds ratio in any other year 7.94 in 2008), see Table 2.3 and Figure 2.2. The odds ratio for 2012 (OR 0.40, CI

0.26-0.62, p<0.001) indicated that the chance of detection of TB was significantly lower, and that for 2014 (OR 1.62, CI 1.28-2.04, p < 0.001) significantly higher than the reference year 2016, although inspection of Figure 2.2 shows the magnitude of these differences to be relatively trivial compared with the much larger differences prior to 2012.

The odds of detecting TB lesions were significantly less in the months of March (OR 0.84, CI 0.74-0.95, p < 0.01), April (OR 0.72, CI 0.63-0.82, p < 0.001), May (OR 0.86, CI 0.71-0.97, p < 0.05), July (OR 0.73, CI 0.65-0.83, p < 0.001), August (OR 0.84, CI 0.74-0.95, p < 0.01), September (OR 0.76, CI 0.67-0.87, p < 0.001), and October (OR , CI 0.75-0.95, p < 0.01) as compared to the reference month of January. The odds for June were also lower (OR 0.94, CI 0.83-1.06, p = 0.291), but not significant. The odds of detecting TB were greater in the months of November (OR 1.27, CI 1.13-1.42), December (OR 1.41, CI 1.27-1.58) and February (OR 1.05, CI 0.93-1.18) compared to the reference month and significantly so (p < 0.001) in November and December but not February (p = 0.44; Table 2.3).

Lesions suggestive of TB were more likely to be detected in the dry seasons (OR 1.37, CI 1.31-1.44, p<0.001) compared to the wet seasons (Table 2.3).

Lesions suggestive of TB were less likely to be detected among female cattle (OR 0.32, CI 0.30-0.34, p<0.001) compared to male cattle (Table 2.3).

Lesions suggestive of TB were significantly more likely to be detected among cattle within the 5 to 10 years old (OR 4.12, CI 3.87-4.38, p<0.001) and >10 years old (OR 5.20, CI 4.85-5.58, p<0.001) age groups compared with the reference category of less than 5 years old age group (Table 2.3).

Lesions suggestive of TB were less likely to be detected among slaughtered cattle from the northern (OR 0.65, CI 0.62-0.69, p<0.001) and southern (OR 0.17, CI 0.14 – 0.20, p<0.001)

geopolitical zones of Plateau State compared with those from the reference central zone (Table 2.3).

Breed was not shown to be associated with the detection of lesions suggestive of TB in slaughtered cattle, having odds ratios of 0.98 (CI 0.92-1.05, p = 0.53) for White Fulani, 0.86 (CI 0.72-1.01, p = 0.073) for Muturu and 1.09 (CI 0.96-1.24, p = 0.194) for mixed breed cattle.

Table 2. 3 Univariable logistic regression analyses of potential risk factors for TB case detection among cattle slaughtered at Jos Abattoir from 2001 to 2016

Variable		Odds Ratio	95% Conf. Interval		p-value
Year					
	2016	REF			
	2001	4.38	3.64	5.28	<0.001
	2002	4.22	3.50	5.07	<0.001
	2003	5.20	4.32	6.26	<0.001
	2004	5.33	4.39	6.46	<0.001
	2005	3.94	3.30	4.70	<0.001
	2006	10.11	8.53	11.99	<0.001
	2007	16.07	13.55	19.06	<0.001
	2008	6.66	5.59	7.94	<0.001
	2009	4.86	4.07	5.80	<0.001
	2010	4.17	3.45	5.03	<0.001
	2011	3.17	2.61	3.84	<0.001
	2012	0.40	0.26	0.62	<0.001
	2013	1.24	0.94	1.64	0.128
	2014	1.62	1.28	2.04	<0.001
	2015	1.04	0.80	1.37	0.749
Month					
	January	REF			
	February	1.05	0.93	1.18	0.441
	March	0.84	0.74	0.95	0.005
	April	0.72	0.63	0.82	<0.001
	May	0.86	0.71	0.97	0.016
	June	0.94	0.83	1.06	0.291
	July	0.73	0.65	0.83	<0.001
	August	0.84	0.74	0.95	0.004
	September	0.76	0.67	0.87	<0.001
	October	0.84	0.75	0.95	0.006
	November	1.27	1.13	1.42	<0.001
	December	1.41	1.27	1.58	<0.001
Season					
	Wet	REF			
	Dry	1.37	1.31	1.44	<0.001
Gender					
	Male	REF			
	Female	0.32	0.30	0.34	<0.001
Age group					
	<5	REF			
	5 to 10	4.12	3.87	4.38	<0.001
	>10	5.20	4.85	5.58	<0.001
Location (geopolitical zone)					
	Central	REF			
	Northern	0.65	0.62	0.69	<0.001
	Southern	0.17	0.14	0.20	<0.001
Breed					
	White Fulani	REF			
	Red Bororo	0.98	0.92	1.05	0.532
	Muturu	0.86	0.72	1.01	0.073
	Mixed Breed	1.09	0.96	1.24	0.194

P<0.05 significant level

2.5.1.4 Multivariable logistic regression analysis of risk factors for the detection of TB in slaughtered cattle

The final multivariable model included the terms year, month, gender, age group, and location. Season was excluded from the multivariable model as it is collinear with month. No evidence of confounding was found for any rejected variables. No statistically significant second-order interactions terms were retained in the final model. Residuals of the final model were clustered close to zero. The results of the Hosmer-Lemeshow test (X-squared = 66.946, df = 8, p-value = $p<0.05$) indicated that there is some evidence of lack of fit to the data, meaning these results should not be used in predictive modelling. However, the results still demonstrate associations over the period of the study between the variables included in the final models and the outcome that was measured.

In the multivariable analysis, lesions suggestive of TB remained significantly more likely to be detected among cattle slaughtered in all years prior to 2012 ($p < 0.001$ for all years 2001 – 2011), compared to the reference year 2016. Over this period (2001 – 2012), the adjusted odds ratios (AOR) ranged between 4.59 (CI 3.78-5.58) in 2011 and 25.20 (CI 21.18 – 29.98) in 2007. As in the univariable analysis, two of these years, 2006 (AOR 14.93, CI 12.56-17.75) and 2007 (AOR 25.20, CI 21.18 – 29.98), showed especially high adjusted odds ratios, with confidence intervals suggesting that the prevalences in these years were significantly higher than those in all other years (highest upper 95% confidence limit for adjusted odds ratio in any other year 10.87 in 2008), see Table 2.3 and Figure 2.2. Again, as in the univariable analysis, the adjusted odds ratio for 2012 (AOR 0.56, CI 0.36-0.86, $p < 0.01$) indicated that the chance of detection of TB was significantly lower than the reference year 2016. The three subsequent years, 2013 (AOR 1.81, CI 1.37-2.40, $p < 0.001$), 2014 (AOR 2.29, CI 1.81-2.89, $p < 0.001$) and 2015 (AOR 1.54, CI 1.17-2.01, $p < 0.01$), all had adjusted odds ratios significantly higher than 2016.

As in the univariable analysis, the magnitude of these post-2011 differences from the reference year were considerably less than the differences in years prior to 2012.

The odds of detecting TB lesions were less likely in the months of March (AOR 0.86, CI 0.75-0.98), April (AOR 0.72, CI 0.63-0.82), May (AOR 0.84, CI 0.73-0.95), July (AOR 0.72, CI 0.63-0.82), August (AOR 0.81, CI 0.72-0.93), September (AOR 0.76, CI 0.66-0.86), and October (AOR 0.85, CI 0.74-0.96) as compared to the month of January, and this was statistically significant. The odds ratio for June was also lower, but that was not significant whereas, the odds of detecting TB lesions was greater in the months of November, December and February compared to the month of January and this was only significant in the months of November and December (Table 2.4).

The odds of detecting lesions suggestive of TB among female cattle was less likely than in male cattle (AOR -0.35, CI 0.32 – 0.38, p= <0.001) (Table 2.4).

The odds of detecting lesions suggestive of TB among cattle within age groups 5 to 10 years old (AOR 5.12, CI 4.80 – 5.46, p <0.001) and greater than 10 years old (AOR 5.78, CI 5.38 – 6.21, p <0.001) were greater than the odds in detecting TB lesions in cattle within less than 5 years old age group (Table 2.4).

The odds of detecting lesions suggestive of TB in cattle from the northern (AOR 0.06, CI 0.56 – 0.63, p= <0.001) and southern zones (AOR 0.28, CI 0.23 – 0.33, p= <0.001) less likely compared to cattle from the central zone respectively (Table 2.4).

Table 2. 4 Multivariable logistic regression analyses of risk factors for TB case detection among cattle slaughtered at Jos Abattoir from 2001 to 2016

Variable		Adjusted Odds Ratio	95% Conf. Interval		p-value
Year					
	2016	REF			
	2001	5.78	4.79	6.98	< 0.001
	2002	5.28	4.37	6.36	< 0.001
	2003	6.96	5.76	8.40	< 0.001
	2004	7.17	5.90	8.71	< 0.001
	2005	5.44	4.55	6.50	< 0.001
	2006	14.93	12.56	17.75	< 0.001
	2007	25.20	21.18	29.98	< 0.001
	2008	9.10	7.62	10.87	< 0.001
	2009	6.55	5.47	7.83	< 0.001
	2010	6.52	5.38	7.89	< 0.001
	2011	4.59	3.78	5.58	< 0.001
	2012	0.56	0.36	0.86	0.008
	2013	1.81	1.37	2.40	< 0.001
	2014	2.29	1.81	2.89	< 0.001
	2015	1.54	1.17	2.01	0.002
Month					
	January	REF			
	February	1.01	0.89	1.14	0.892
	March	0.86	0.75	0.98	0.021
	April	0.72	0.63	0.82	< 0.001
	May	0.84	0.73	0.95	0.006
	June	0.98	0.86	1.11	0.752
	July	0.72	0.63	0.82	< 0.001
	August	0.81	0.72	0.93	0.002
	September	0.76	0.66	0.86	< 0.001
	October	0.85	0.74	0.96	0.011
	November	1.33	1.18	1.50	< 0.001
	December	1.47	1.31	1.65	< 0.001
Gender					
	Male	REF			
	Female	0.35	0.32	0.38	< 0.001
Age group					
	<5	REF			
	5 to 10	5.12	4.80	5.46	< 0.001
	>10	5.78	5.38	6.21	< 0.001
Location (geopolitical zone)					
	Central	REF			
	North	0.06	0.56	0.63	< 0.001
	South	0.28	0.23	0.33	< 0.001

P<0.05 significant level

2.5.2 Human Tuberculosis in Jos University Teaching Hospital 2001-2015

2.5.2.1 Data characteristics (human dataset)

The data contain records of a total of 47,173 patients tested for TB from 2001 to 2015. The highest number of people tested for TB was in 2012 (n=3,908, 8.28%), in the months of December (n=5,146, 10.91%), in the wet season (n=24,193, 51.29%) and among those from the central geopolitical zone of the state (n=20176, 42.77%). The lowest number of people tested for TB was in the year 2007 (n=2,009, 4.26%) and in the month of August (n=3,087, 6.54%).

The modal age group of people tested for TB was 21-40 years old (n=20,462, 43.38%) and the age group with the fewest people tested for TB was above 60 years old (n=2,303, 4.88%). Overall, males accounted for slightly more than half (n= 23,918, 50.70%) of the people tested for TB (Table 2.5).

Table 2. 5 Characteristic of human dataset

		Frequency	Proportion (%)	95% Conf. Interval	
Year					
	2001	2046	4.34	4.16	4.52
	2002	2086	4.42	4.24	4.61
	2003	3001	6.36	6.14	6.59
	2004	3045	6.45	6.24	6.68
	2005	3398	7.2	6.97	7.44
	2006	3456	7.33	7.09	7.56
	2007	2009	4.26	4.08	4.44
	2008	3600	7.63	7.40	7.87
	2009	3578	7.58	7.35	7.83
	2010	3028	6.42	6.20	6.64
	2011	3412	7.23	7.00	7.47
	2012	3908	8.28	8.04	8.54
	2013	2987	6.33	6.12	6.56
	2014	3720	7.89	7.65	8.13
	2015	3899	8.27	8.02	8.52
Month					
	January	4462	9.46	9.20	9.73
	February	4634	9.82	9.56	10.1
	March	4271	9.05	8.80	9.32
	April	3482	7.38	7.15	7.62
	May	3180	6.74	6.52	6.97
	June	3367	7.14	6.91	7.37
	July	3088	6.55	6.33	6.77
	August	3087	6.54	6.32	6.77
	September	3971	8.42	8.17	8.67
	October	4018	8.52	8.27	8.77
	November	4467	9.47	9.21	9.74
	December	5146	10.91	10.63	11.19
Season					
	Wet	24193	51.29	50.83	51.74
	Dry	22980	48.71	48.26	49.17
Gender					
	Male	23918	50.7	50.25	51.15
	Female	23255	49.3	48.85	49.75
Age-group					
	<20	14679	31.12	30.70	31.54
	21 to 40	20462	43.38	42.93	43.82
	41 to 60	9729	20.62	20.26	20.99
	>60	2303	4.88	4.69	5.08
Location (Geopolitical zones)					
	Central	20176	42.77	42.32	43.22
	Northern	12297	26.07	25.67	26.47
	Southern	14700	31.16	30.75	31.58
Total		**47173**			

2.5.2.2 Prevalence of TB in humans tested for TB at Jos University Teaching Hospital

From 2001-2015, a total of 47,173 people was tested for TB at JUTH of which 6,709 were positive, giving an overall prevalence of 14.22% (CI 13.91-14.54%). The annual mean number of TB cases in humans was 447.3 and the median was 459.

The number of human TB cases varied very highly significantly across the years of the study (χ^2=285.23, P= <0.001). The lowest number of TB cases (n=307) was seen in 2007 and the highest (n=589) in 2003. The lowest TB prevalence in humans (10.34%, CI 9.30-1149%) was seen in 2013 and the highest prevalence (19.63%, CI 18.25-21.09%) was seen in 2003 (Table 2.6, Figure 2.9).

The number of TB cases was shown to vary with very high statistical significance across calendar months (χ^2=322.34, P= <0.001). The month of June showed the lowest number of TB cases in humans (n=334) while highest number was in January (n=933). Likewise, the lowest TB prevalence in humans was seen in June (10.37%, CI 9.38 – 11.44) and the highest in January (20.91%, CI19.74 – 22.13) (Table 2.6, Figure 2.12).

There were significantly more TB cases in humans during the dry seasons (3,748 cases, n=22,980) than in the wet seasons (2,961cases, n=24,193; χ^2=160.09, P= <0.001). Accordingly, the wet season had a lower TB prevalence (12.24%, CI 11.83-12.66) than the dry season (prevalence 16.31%, CI 15.84-16.79) (Table 2.6, Figure 2.11 and 2.12).

The number of TB cases did not differ significantly between the two genders (χ^2=1.31, P= 0.253). There were similar numbers of TB cases in males (3,445 cases, n=23,918) and females (3,264 cases, n=23,255) and correspondingly similar prevalences of 14.40% (CI 13.96-14.86) and 14.04% (CI 13.60-14.49) respectively (Table 2.6, Figure 2.13).

The number of TB cases varied highly significantly among age groups (χ^2=492.93, P <0.001). Patients within age group 21-40 years old had the highest number of TB cases (n= 3,668) and

those within age groups greater than 60 years old had the lowest cases of TB (n= 145). Patients within age groups 21-40 years old had the highest TB prevalence (17.93%, CI 17.41-18.46%) and the lowest TB prevalence (6.30%, CI 5.38-7.36%) was seen among patients within age group above 60 years old (Table 2.6. Figure 2.14).

TB case varied significantly across the three geopolitical zones (χ^2=79.52, P<0.001). Patients from the central zone had the highest number of TB cases (n=2,597) and those from northern zone had the lowest number of TB cases (n=2,021). Patients from the northern zone had the highest prevalence rate (16.43%, CI 15.79-17.10%) and the lowest TB prevalence was seen among patients from the central zones (12.87%, CI 12.42-13.34%; Table 2.6, Figure 2.15).

Table 2. 6 Prevalence and its 95% confidence intervals of TB in humans among those tested from 2001- 2015 and Chi-squared test of association between TB case in humans and year, age group, gender, geopolitical zone (location), season and month

		No. of People tested	No. of TB cases	Prevalence (%)	95% Conf. Interval (%)		Chi-squared	P-value
Year							285.23	<0.001
	2001	2,046	357	17.45	15.87	19.15		
	2002	2,086	400	19.18	17.54	20.92		
	2003	3,001	589	19.63	18.25	21.09		
	2004	3,045	526	17.27	15.97	18.66		
	2005	3,398	530	15.6	14.42	16.86		
	2006	3,456	498	14.41	13.28	15.62		
	2007	2,009	307	15.28	13.77	16.92		
	2008	3,600	518	14.39	13.28	15.57		
	2009	3,578	459	12.83	11.77	13.96		
	2010	3,028	380	12.55	11.42	13.78		
	2011	3,412	461	13.51	12.41	14.7		
	2012	3,908	550	14.07	13.02	15.2		
	2013	2,987	309	10.34	9.30	11.49		
	2014	3,720	414	11.13	10.16	12.18		
	2015	3,899	411	10.54	9.62	11.54		
Month							322.34	<0.001
	January	4,462	933	20.91	19.74	22.13		
	February	4,634	782	16.88	15.82	17.98		
	March	4,271	691	16.18	15.11	17.31		
	April	3,482	390	11.2	10.20	12.29		
	May	3,180	393	12.36	11.26	13.55		
	June	3,367	349	10.37	9.38	11.44		
	July	3,088	392	12.69	11.57	13.92		
	August	3,087	334	10.82	9.77	11.96		
	September	3,971	585	14.73	13.66	15.87		
	October	4,018	518	12.89	11.89	13.96		
	November	4,467	626	14.01	13.03	15.06		
	December	5,146	716	13.91	13.00	14.89		
Season							160.09	<0.001
	Wet	24,193	2961	12.24	11.832	12.658		
	Dry	22,980	3748	16.31	15.838	16.793		
Gender							1.31	0.253
	Male	23,918	3445	14.4	13.964	14.854		
	Female	23,255	3264	14.04	13.595	14.488		
Age group							492.93	<0.001
	<20	14,679	1588	10.82	10.326	11.331		

	21 to 40	20,462	3668	17.93	17.406	18.457		
	41 to 60	9,729	1308	13.44	12.781	14.137		
	>60	2,303	145	6.3	5.375	7.363		
Location (geopolitical zones)							79.52	<0.001
	Central	20,176	2597	12.87	12.417	13.341		
	Northern	12,297	2021	16.43	15.79	17.1		
	Southern	14,700	2091	14.22	13.669	14.799		
Total		**47,173**	**6,709**	**14.22**	**13.91**	**14.54**		

P<0.05 significant level

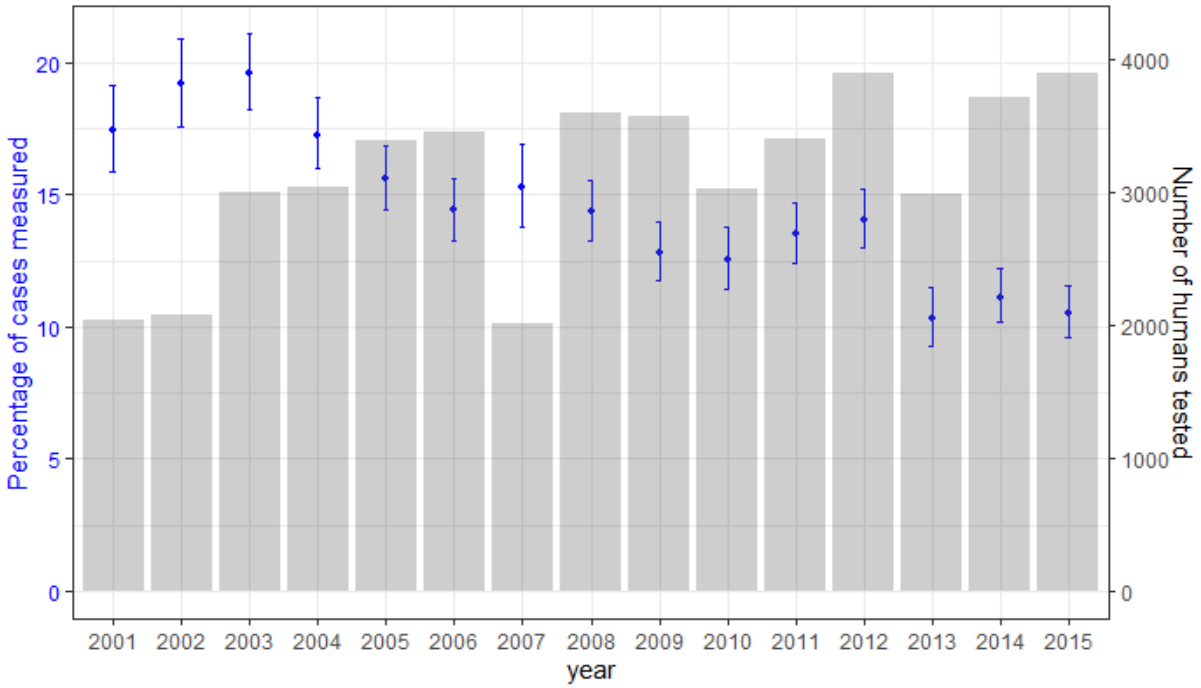

Figure 2. 9 Number of patients tested for TB in each year (grey bars) and the prevalence of TB in these patients (blue symbols) with 95% confidence intervals (blue error bars)

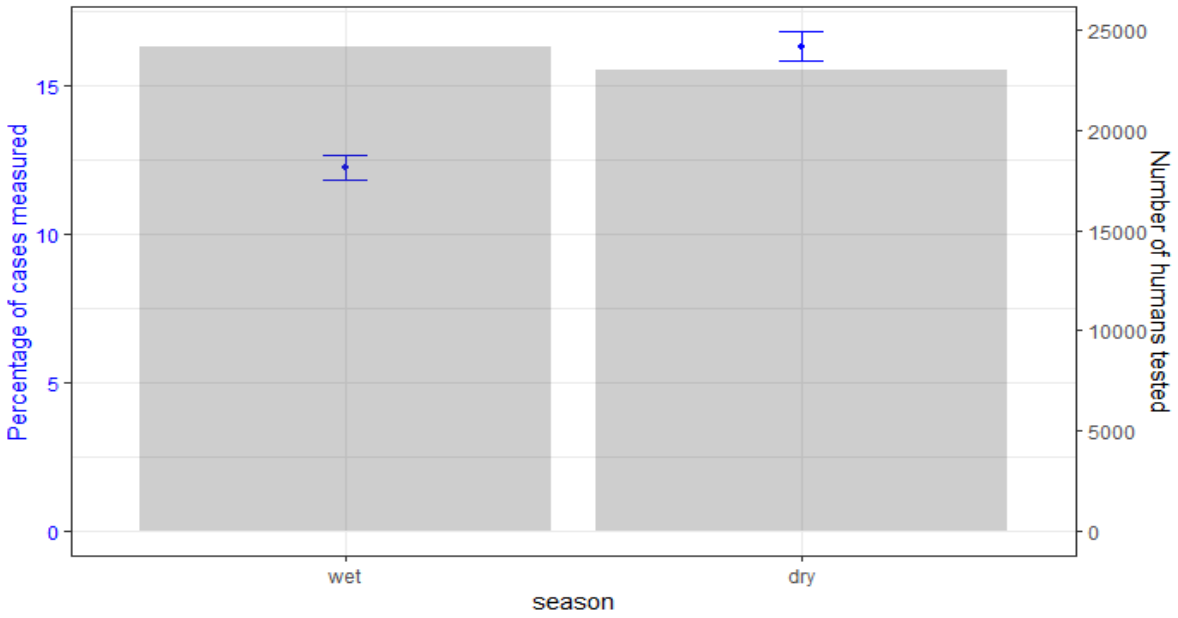

Figure 2. 10 Number of patients tested for TB in each season (grey bars) and the prevalence of TB in these patients (blue symbols) with 95% confidence intervals (blue error bars)

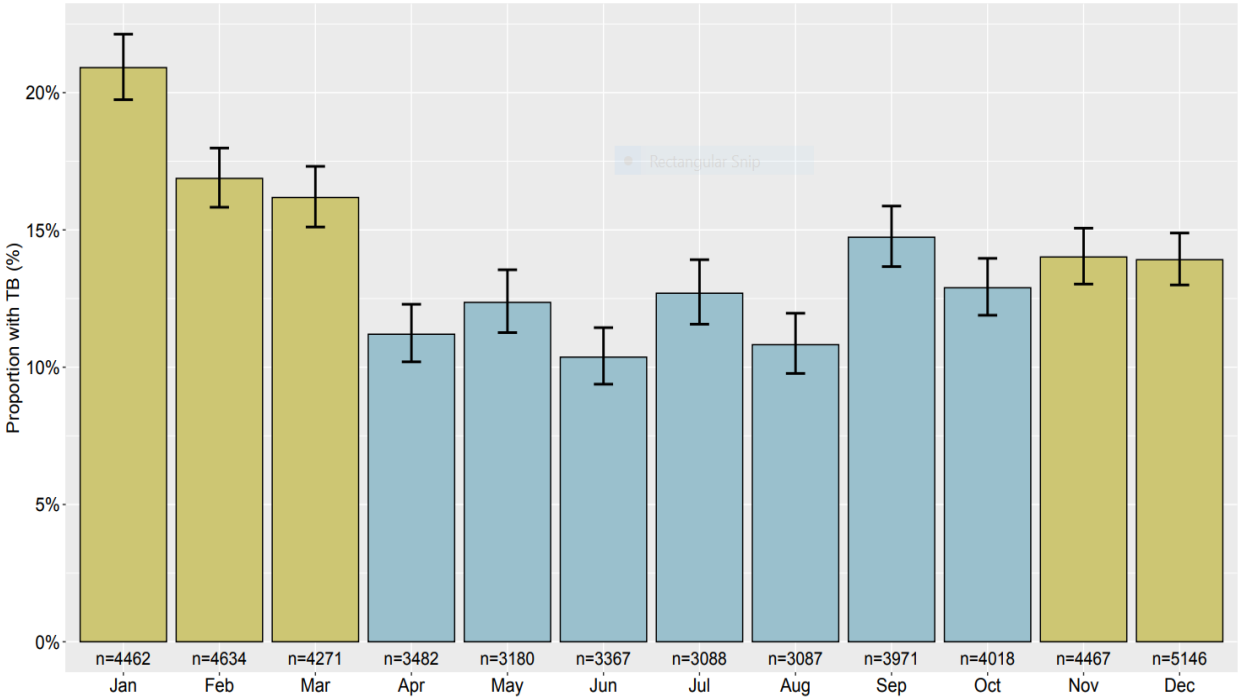

Figure 2. 11 Average monthly prevalence of TB in patients tested at Jos University Teaching Hospital 2001-2015. Khaki bars: dry season; light blue bars: wet season. Error bars: 95% confidence intervals. Number of patients tested shown below each bar

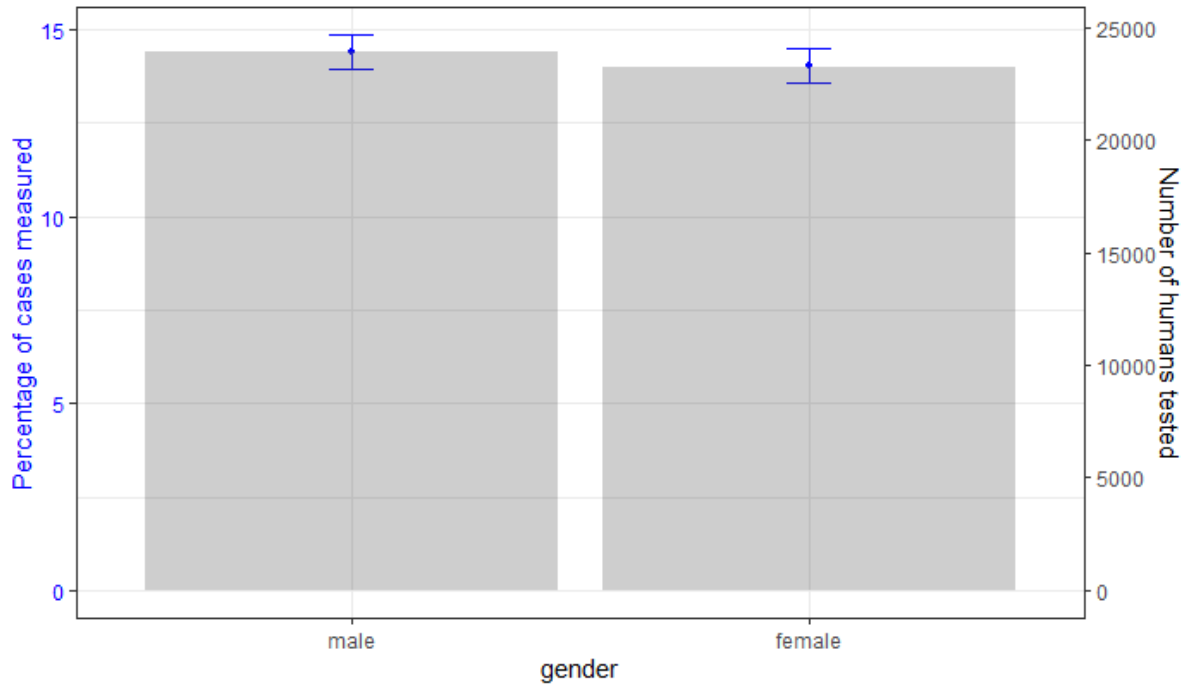

Figure 2. 12 Number of male and female patients tested for TB (grey bars) and the prevalence of TB in these patients (blue symbols) with 95% confidence intervals (blue error bars)

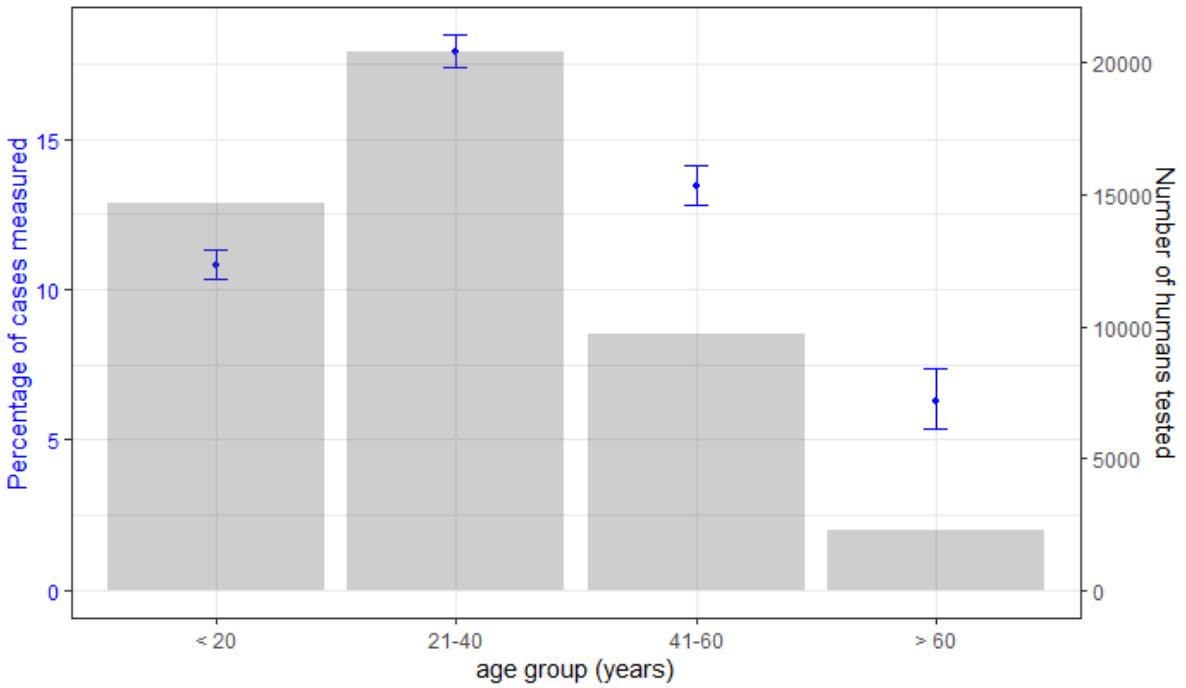

Figure 2. 13 Number of patients in each age group tested for TB (grey bars) and the prevalence of TB in these patients (blue symbols) with 95% confidence intervals (blue error bars)

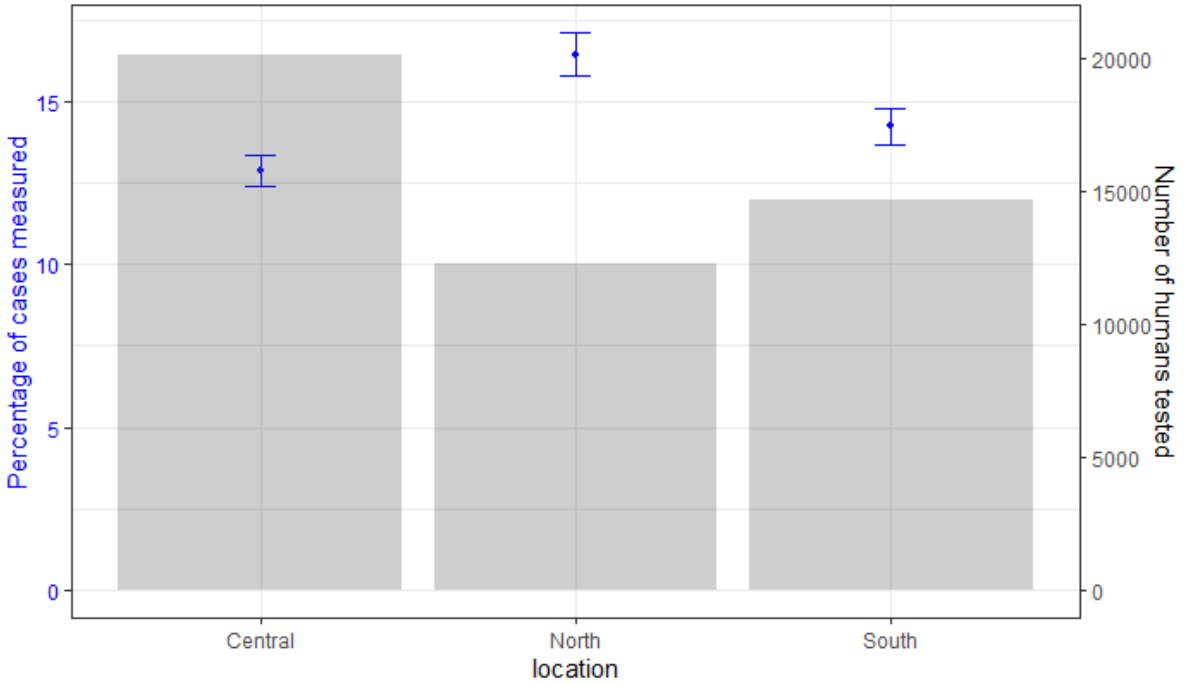

Figure 2. 14 Number of patients in each geopolitical zone tested for TB (grey bars) and the prevalence of TB in these patients (blue symbols) with 95% confidence intervals (blue error bars)

2.5.2.3 Univariable logistic regression analysis of risk factors for the detection of TB in humans tested for TB at Jos University Teaching Hospital

Univariable logistic regression analysis showed that the following variables were associated with positive test among patients tested for TB at JUTH: year, month, season, age group, and location.

During the first twelve years of the study (2001 to 2012), the odds of a positive test among patients tested for TB was significantly greater ($p < 0.01$) than those in the reference year 2015, with odds ratios ranging from 1.22 (CI 1.05-1.41, $p < 0.01$) in 2010 to 2.07 (CI 1.81-2.38, $p < 0.001$) in 2003. For the last two years (2013, 2014) prior to the reference year, the differences were not significant (2013: OR 0.98, CI 0.84-1.14, P= 0.792; 2014: OR 1.06, CI 0.92-1.23, $p = 0.409$; Table 2.7).

The odds of a positive test among patients tested for TB were significantly lower in the months between February and December than in the reference month of January, with odds ratios ranging from 0.44 (CI 0.38-0.50, $p < 0.001$) in June to 0.77 (CI 0.69-0.85, $p < 0.001$) in February (Table 2.7).

The odds of a positive TB test among patients during the dry seasons (OR 1.40, CI 1.33 – 1.47, $p<0.001$) was significantly greater than in the reference wet season (Table 2.7).

The odds of a positive TB test among female patients (OR 0.97, CI 0.92 – 1.02, P=0.253) was less than among male patients but this difference was not significant (Table 2.7).

Patients within age groups 21-40 (OR 1.80, CI 1.69 – 1.92, $p<0.001$) and 41-60 (OR 1.28, CI 1.18 – 1.39, $p<0.001$) years old had greater odds of a positive TB test than patients within the reference age group of ≤ 20 years old. Patients above 60 years of age were less likely (OR 0.55,

CI 0.47 – 0.66, p<0.001) to get a positive TB result than patients within the reference age group (Table 2.7).

Patients from the northern (OR 1.33, CI 1.25 – 1.42, p<0.001) and southern (OR 1.12, CI 1.06 – 1.19, p<0.001) zones of the state had greater odds of a positive TB test result compared to patients from the reference central zone (Table 2.7).

Table 2. 7 Univariable logistic regression analyses of potential risk factors for a positive result among humans tested for TB at Jos University Teaching Hospital from 2001 to 2015

Variable		Odds Ratio	95% Conf. Interval		p-value
Year					
	2015	REF			
	2001	1.79	1.54	2.09	<0.001
	2002	2.01	1.73	2.34	<0.001
	2003	2.07	1.81	2.38	<0.001
	2004	1.77	1.54	2.04	<0.001
	2005	1.57	1.37	1.80	<0.001
	2006	1.43	1.24	1.64	<0.001
	2007	1.53	1.31	1.79	<0.001
	2008	1.43	1.24	1.64	<0.001
	2009	1.25	1.08	1.44	0.002
	2010	1.22	1.05	1.41	0.009
	2011	1.33	1.15	1.53	<0.001
	2012	1.39	1.21	1.59	<0.001
	2013	0.98	0.84	1.14	0.792
	2014	1.06	0.92	1.23	0.409
Month					
	January	REF			
	February	0.77	0.69	0.85	<0.001
	March	0.73	0.65	0.81	<0.001
	April	0.48	0.42	0.54	<0.001
	May	0.53	0.47	0.61	<0.001
	June	0.44	0.38	0.50	<0.001
	July	0.55	0.48	0.63	<0.001
	August	0.46	0.40	0.52	<0.001
	September	0.65	0.58	0.73	<0.001
	October	0.56	0.50	0.63	<0.001
	November	0.62	0.55	0.69	<0.001
	December	0.61	0.55	0.68	<0.001
Season					
	Wet	REF			
	Dry	1.40	1.33	1.47	<0.001
Gender					
	Male	REF			
	Female	0.97	0.92	1.022	0.253
Age group					
	≤20	REF			
	21 to 40	1.80	1.69	1.92	<0.001
	41 to 60	1.28	1.18	1.39	<0.001
	>60	0.55	0.47	0.66	<0.001
Location (geopolitical zone)					
	Central	REF			
	Northern	1.33	1.25	1.42	<0.001
	Southern	1.12	1.06	1.19	<0.001

P<0.05 significant level

2.5.2.4 Multivariable logistic regression analysis of risk factors for the detection of TB in humans tested for TB at JUTH

The final multivariable model included the terms year, month, age group and location. Season was excluded from the multivariable model as it is colinear with month. Gender was not included in the multivariable model as it was not significant in the univariable analysis.

No evidence of confounding was found for any rejected variables. No statistically significant second-order interactions terms were retained in the final model. Residuals of the final model were clustered close to zero. The results of the Hosmer-Lemeshow test (X-squared = 73.089, df = 8, p-value = <0.05) indicate that there is some evidence of lack of fit to the data, meaning these results should not be used in predictive modelling. However, the results still demonstrate associations over the period of the study between the variables included in the final models and the outcome that was measured.

The odds of patients testing positive for TB at JUTH in each year of the study were broadly similar in the multivariable analysis to those obtained in the univariable analysis. All years prior to 2013 had significantly greater odds ($p < 0.01$) of patients testing positive than in the reference year (2015), with adjusted odds ratios ranging from 1.28 (CI 1.10-1.48, $p < 0.01$) in 2009 to 2.46 (CI 2.13-2.84, $p < 0.001$) in 2003. Again, as in the univariable analysis, for the last two years (2013, 2014) prior to the reference year the differences were not significant (2013: AOR 0.89, CI 0.76-1.05, P= 0.164; 2014: AOR 0.98, CI 0.85-1.14, p = 0.799; Table 2.8).

As in the univariable analysis, the odds of a positive test among patients tested for TB were significantly lower in the months between February and December than in the reference month of January, with adjusted odds ratios ranging from 0.43 (CI 0.38-0.50, $p < 0.001$) in June to 0.74 (CI 0.67-0.83, $p < 0.001$) in February (Table 2.8).

Patients within the age groups 21-40 (AOR 1.74, CI 1.63 – 1.86, p<0.001) and 41-60 (AOR 1.32, CI 1.22 – 1.44, p<0.001) years old had greater odds of a positive TB test than patients within the reference age group of ≤ 20 years old. Patients that were within the above 60 years old age group were (AOR 0.58, CI 0.48 – 0.69, p<0.001) less likely to test positive than patients within the reference age group (Table 2.8).

Patients from the northern (AOR 1.70, CI 1.59 – 1.83, p<0.001) and southern (AOR 1.44, CI 1.35 – 1.54, p<0.001) zones of the state had greater odds of a positive TB test results than patients from the reference central zone (Table 2.8).

Table 2. 8 Multivariable logistic regression analyses of risk factors for TB among humans tested from 2001 to 2015

Variable		Adjusted Odds Ratio	95% Conf. Interval		p-value
Year					
	2015	REF			
	2001	1.94	1.66	2.28	< 0.001
	2002	2.23	1.91	2.61	< 0.001
	2003	2.46	2.13	2.84	< 0.001
	2004	2.06	1.78	2.38	< 0.001
	2005	1.80	1.56	2.08	< 0.001
	2006	1.51	1.30	1.74	< 0.001
	2007	1.45	1.23	1.71	< 0.001
	2008	1.42	1.23	1.64	< 0.001
	2009	1.28	1.10	1.48	0.001
	2010	1.41	1.21	1.64	< 0.001
	2011	1.59	1.38	1.84	< 0.001
	2012	1.47	1.28	1.69	< 0.001
	2013	0.89	0.76	1.05	0.164
	2014	0.98	0.85	1.14	0.799
Month					
	January	REF			
	February	0.74	0.67	0.83	< 0.001
	March	0.71	0.63	0.79	< 0.001
	April	0.46	0.40	0.52	< 0.001
	May	0.52	0.46	0.60	< 0.001
	June	0.43	0.38	0.50	< 0.001
	July	0.54	0.47	0.61	< 0.001
	August	0.44	0.38	0.50	< 0.001
	September	0.65	0.58	0.73	< 0.001
	October	0.55	0.49	0.62	< 0.001
	November	0.60	0.53	0.67	< 0.001
	December	0.62	0.55	0.69	< 0.001
Age group					
	≤ 20	REF			
	21-40	1.74	1.63	1.86	< 0.001
	41-60	1.32	1.22	1.44	< 0.001
	> 60	0.58	0.48	0.69	< 0.001
Location (geopolitical zone)					
	Central	REF			
	Northern	1.70	1.59	1.83	< 0.001
	Southern	1.44	1.35	1.54	< 0.001

P<0.05 significant level

2.5.3 Seasonal-trend decomposition

Untransformed monthly TB prevalence data showed marked and significant positive skewness for both abattoir cattle (skewness 1.50, se 0.177, $t = 8.48$, d.f. 191, $p < 0.001$) and hospital patients (skewness 0.744, se 0.183, $t = 4.08$, d.f. 191, $p < 0.001$), whereas Box-Cox transformations with optimised lambda values of 0.1921 and 0.6849 for cattle and human data respectively reduced skewness to an absolute magnitude of less than 0.0001 ($p = 0.500$) in both cases (Figures 2.16 and 2.17). The corresponding untransformed and Box-Cox transformed time-series plots for abattoir cattle and hospital patient TB monthly prevalence data are shown in Figures 2.18 and 2.19 respectively.

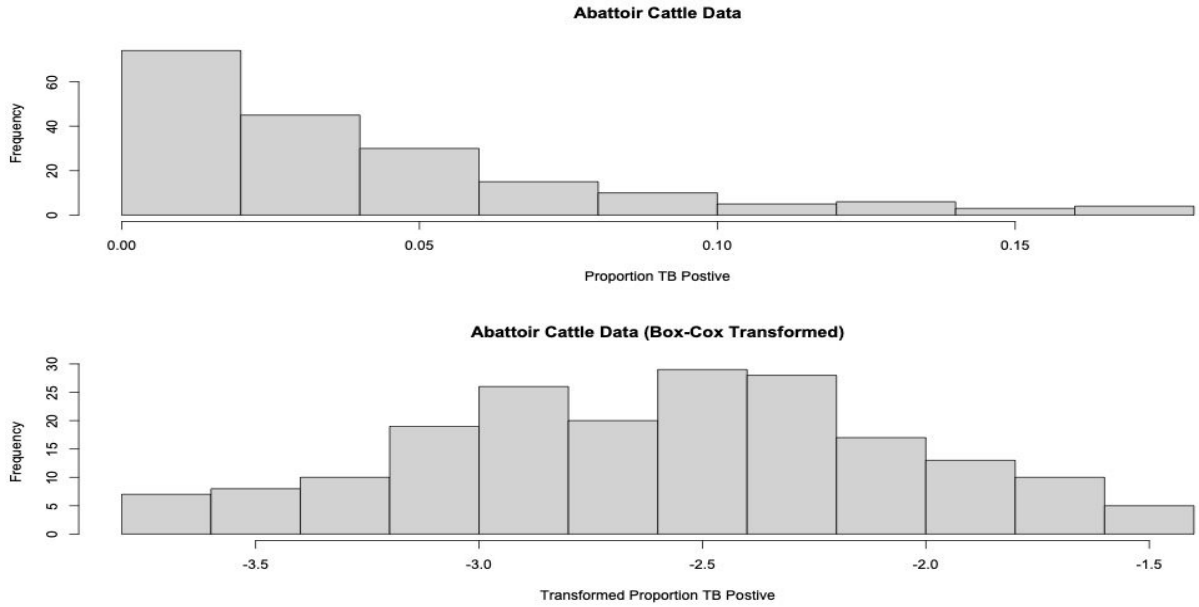

Figure 2. 15 Histograms of untransformed monthly TB prevalence data (upper graph) and Box-Cox transformed data (lower graph) of TB cases in cattle slaughtered at the abattoir from January 2001 until December 2016

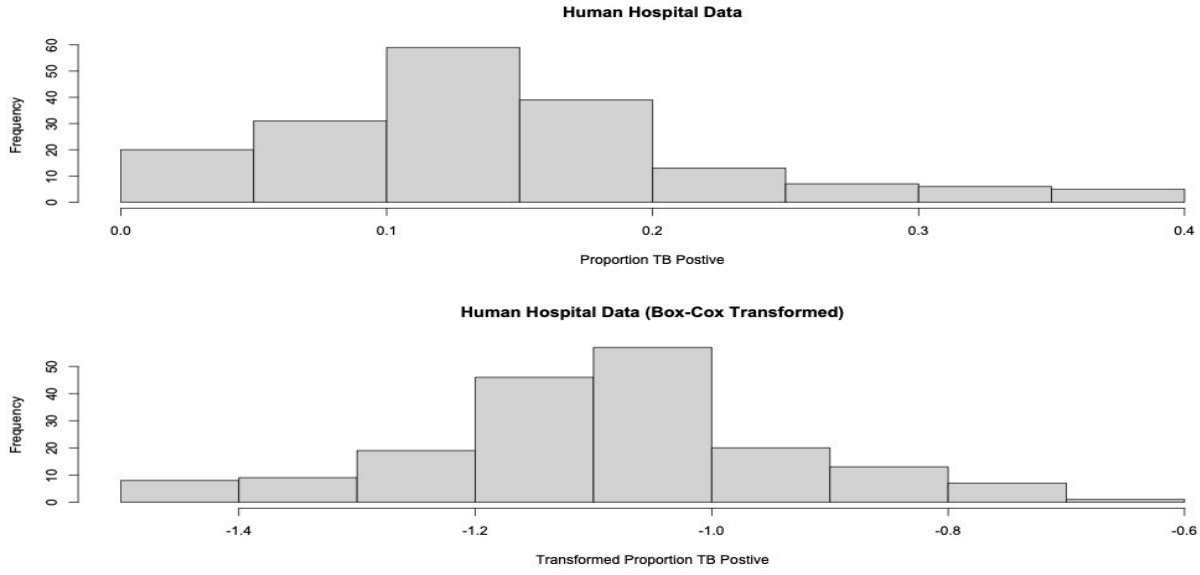

Figure 2. 16 Histograms of untransformed monthly TB prevalence data (upper graph) and Box-Cox transformed data (lower graph) of TB cases in humans tested at JUTH from January 2001 until December 2015

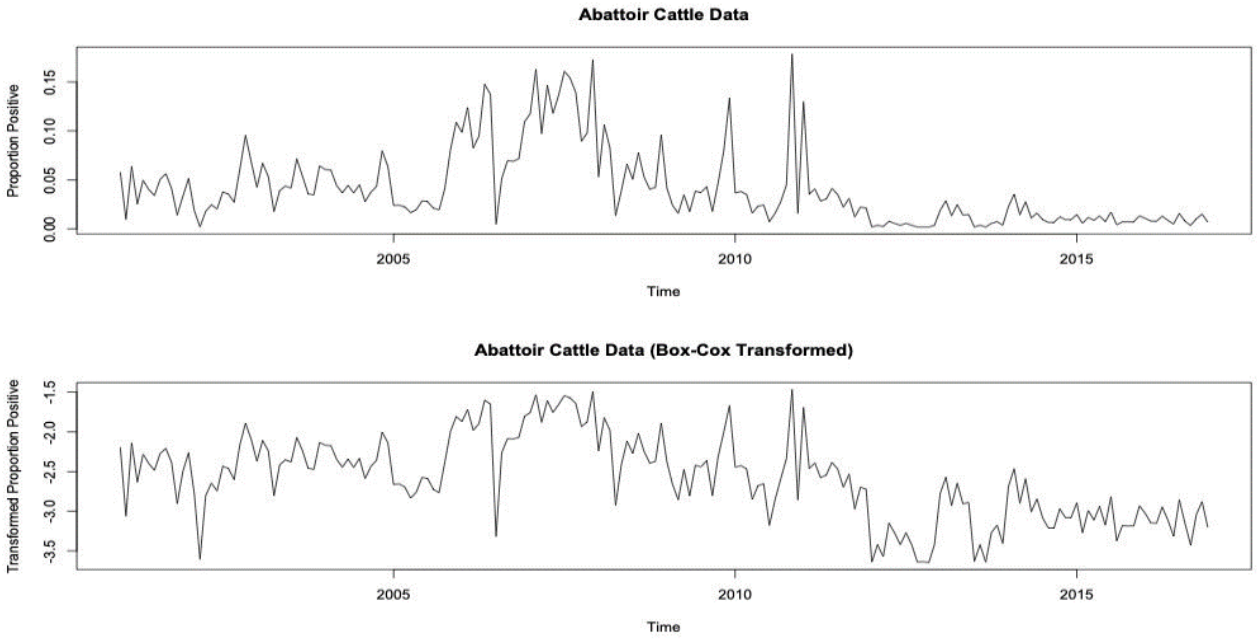

Figure 2. 17 Time series plot untransformed monthly TB prevalence data (upper graph) and Box-Cox transformed data (lower graph) of TB cases in cattle slaughtered at the abattoir from January 2001 until December 2016

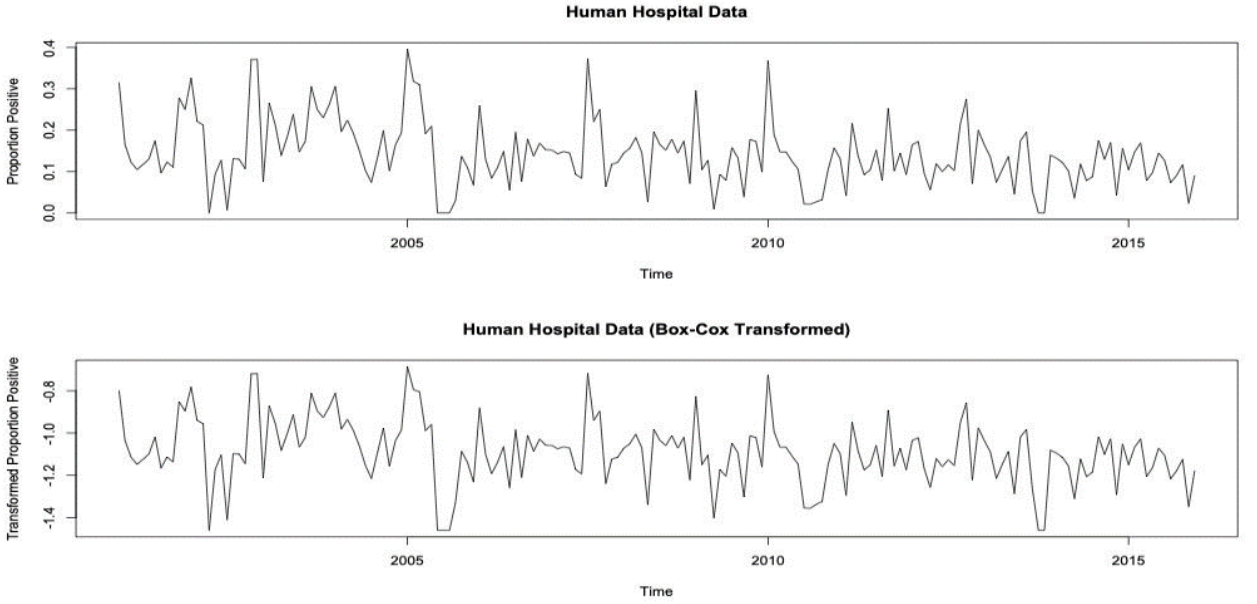

Figure 2. 18 Time series plot untransformed monthly TB prevalence data (upper graph) and Box-Cox transformed data (lower graph) of TB cases in humans tested at JUTH from January 2001 until December 2015

Seasonal-trend decompositions based on loess (STL) plots of both the Box-Cox transformed abattoir cattle and hospital patient TB monthly prevalence data are shown in Figures 2.20 and 2.21. Both cattle and human data STL plots showed strong seasonal peaks in December and January respectively, and somewhat irregular troughs over the intervening wet season months (April-October). The trend in the cattle abattoir data suggested four phases: 1. a reasonably steady state over the period 2001 - 2005; 2. a marked increase in 2006, peaking in 2007; 3. a return to the phase 1 (2001-2005) levels over the period 2008 to mid-2012; and 4. a marked fall in level in mid-2012 followed by a new lower steady state until the end of 2016. The multivariable logistic regression analysis for TB in slaughtered cattle was re-run replacing the term for year with a factor having four levels representing these four phases (respectively 2001-2005; 2006-2007; 2008-2011; and 2012-2016). Using the first phase as the reference level, all three subsequent phases differed from it significantly ($p < 0.001$), having odds ratios of 3.17 (CI 2.97–3.39), 1.13 (CI 1.06–1.21) and 0.225 (CI 0.203–0.249) respectively. This model (AIC 46,733) was however a poorer fit overall than the original model having a distinct level for each year (AIC 46441).

The trend in the human hospital data was less clear but suggested an overall decline over the 15-year period. This decline was supported both by a simple linear model of the STL trend over time (intercept -0.910, slope -0.0119 per year, adjusted R-squared 0.616, $P < 0.001$) and a generalised linear model of the untransformed time series data with TB status as the binary outcome variable and year and month as explanatory variables, giving an odds ratio of 0.953 (CI 0.947–0.959, $p < 0.001$) per year.

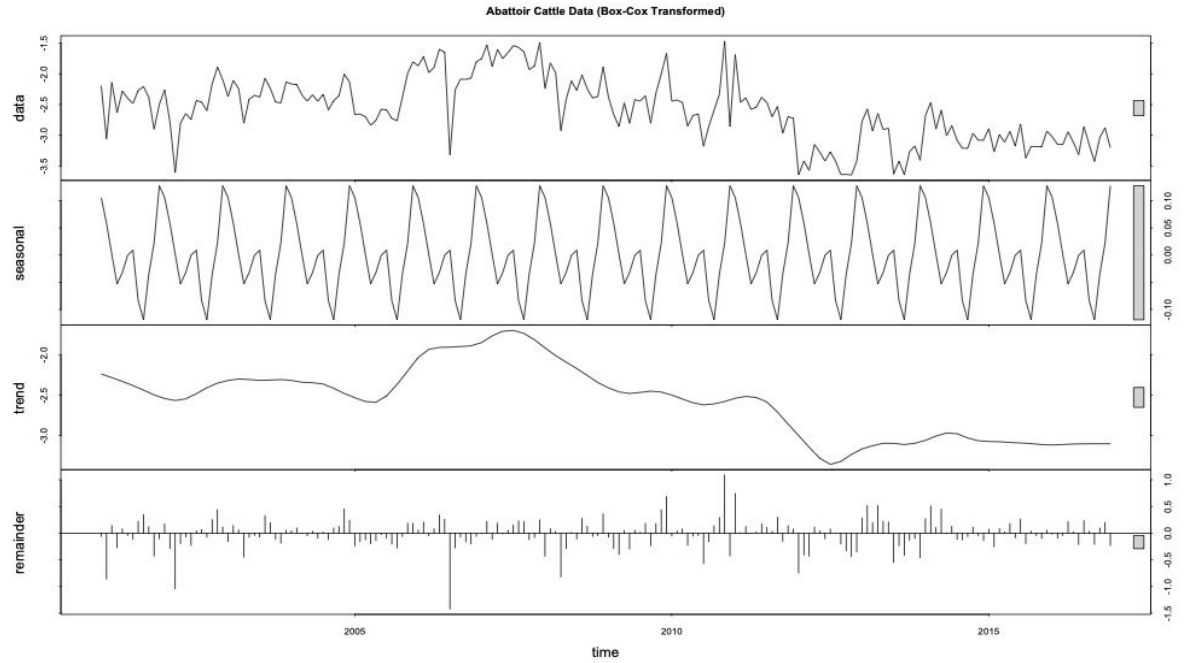

Figure 2. 19 Seasonal time series decomposition of monthly TB cases in cattle slaughtered at the Jos abattoir from January 2001 to December 2016 showing different deconstructed components (Data, Seasonality, Trend and Remainder) of the TB series with the STL method

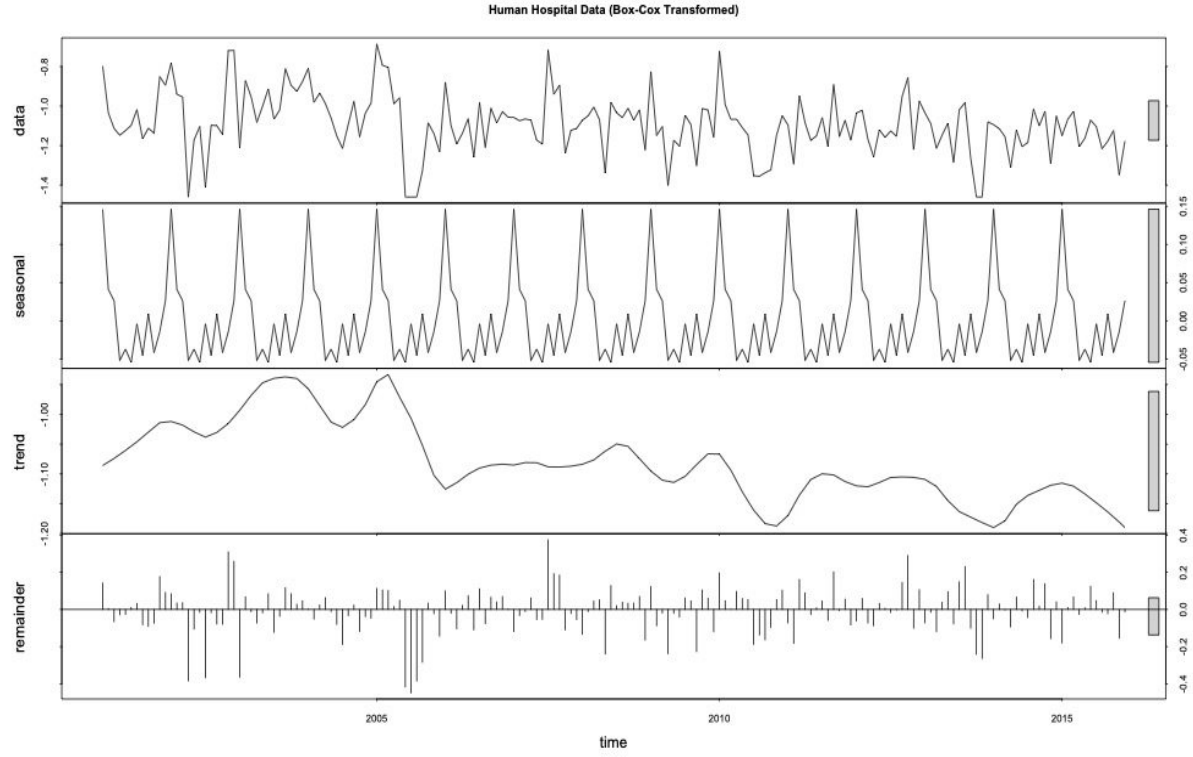

Figure 2. 20 Seasonal time series decomposition of monthly TB cases in humans tested at JUTH from January 2001 to December 2015 showing different deconstructed components (Data, Seasonality, Trend and Remainder) of the TB series with the STL method

The autocorrelation and partial autocorrelation of the residuals of the STL of the Box-Cox transformed abattoir cattle and hospital patient TB monthly prevalence data are shown in Figures 2.22 and 2.23 These correlograms show little evidence of autocorrelation from the cattle data and none for the human data. For the cattle data, some of the spikes in the autocorrelation and partial autocorrelation plots do marginally cross the expected limits. These are mainly a very slight positive correlation at one-month, slight negative correlation around 6 months and a slight positive correlation at 18 months, all of which could be accounted for by "leakage" of the seasonal cycles not fully captured by the STL procedure. However, there is no convincing evidence of correlation between consecutive years.

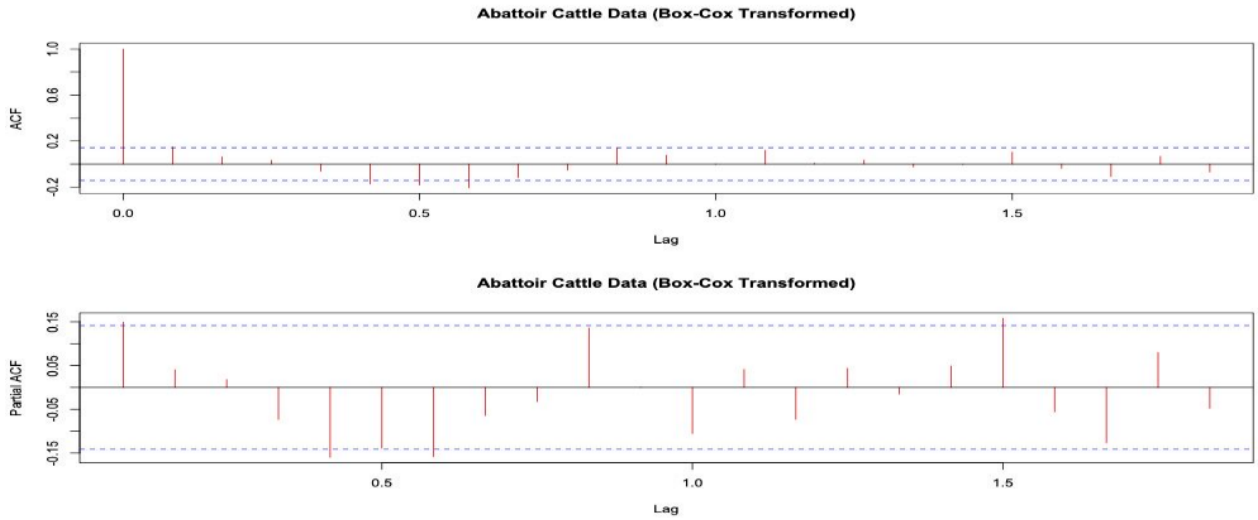

Figure 2. 21 Correlogram plot of the ACF (upper Graph) and PACF (lower Graph) for the log-transformed of actual cattle data at various lags. The horizontal dash lines in the ACF and PACF are the significant bounds

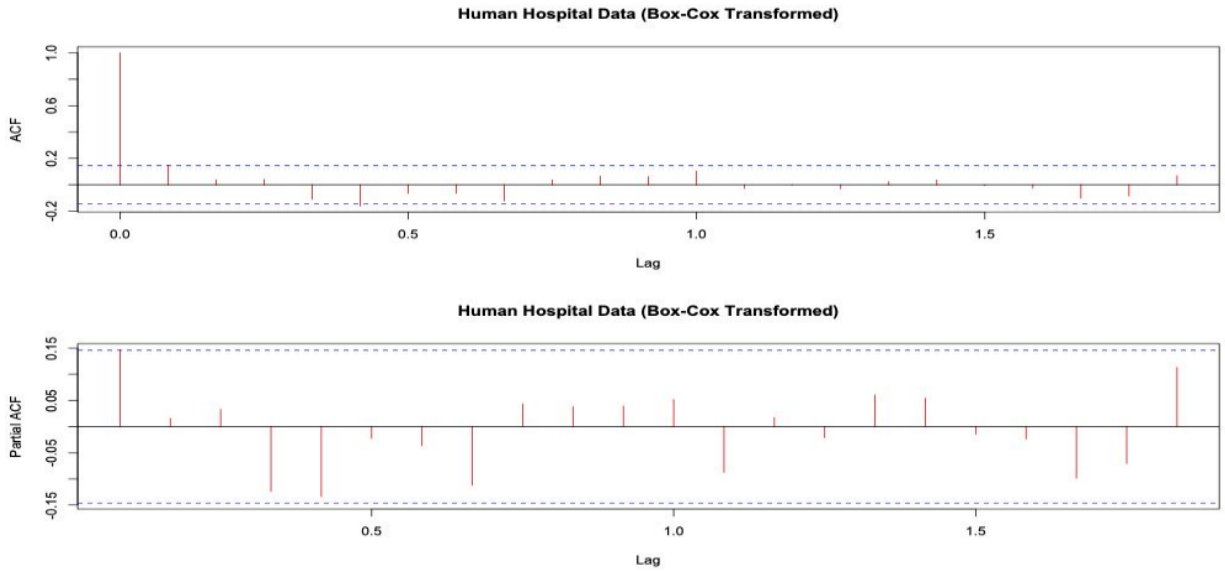

Figure 2. 22 Correlogram plot of the ACF (upper Graph) and PACF (lower Graph) for the log-transformed of the actual human data at various lags. The horizontal dash lines in the ACF and PACF are the significant bounds

2.6 Discussion

Given the recognised presence of TB in cattle in Nigeria (Abubakar et al., 2011a; Adang et al., 2015; Agbalaya et al., 2020; Ibrahim et al., 2010), the importance of cattle production in Plateau State, the close relationship of animals and humans, including risky behaviours and practices such as co-habitation with livestock and consumption of diseased meat and unpasteurized milk, the likelihood of zoonotic TB in Nigeria was considered to be an important cause of concern for public health. Investigation of historical prevalences of TB in both cattle and humans, and of associated risk factors, was undertaken using retrospective data on cattle presented for slaughter at Jos Abattoir and humans tested for TB at Jos University Teaching Hospital.

Cattle

A retrospective review of abattoir records on cattle slaughtered and number of cattle with lesions suggestive of TB showed an overall TB prevalence of 4.0% which is high when compared to reports of prevalence rates in retrospective studies on bovine tuberculosis in abattoirs in some states in Nigeria of 1.9% reported by Ejeh et al. (2014b) in Makurdi, 2.7% by Abubakar et al. (2011b) in Maiduguri, 1.13% by Jajere et al. (2018) in Bauchi, 1.40% by Nwanta et al. (2011) in Enugu, 0.72% by Akinbobola et al. (2017) in Abuja and 0.78% by Adamu et al. (2017) in Gombe State. It is however lower than the 6.7% prevalence rate reported by Danbirni et al. (2013) in Adamawa State.

The highest prevalences of TB in cattle seen in 2006 (8.8%) and 2007 (13.3%) were similar to the findings of Okeke et al. (2016) in Plateau State, who suggested the reason for the apparently high prevalence in that period could be more thorough meat inspection and disease reporting in the state in response to an outbreak of highly pathogenic avian influenza (Fusaro et al, 2009). Similarly, the apparently low prevalence seen in 2012 (0.38%), and to some extent in subsequent years (all prevalences 2012-2016 < 2%), may have been due to religious and ethnic

crises at that time which led to insecurity and low attendance rates of staff in the abattoir and underreporting of disease (Ukwayi et al, 2018).

The prevalence of TB in cattle was significantly higher (univariable analysis $p < 0.001$) in the dry seasons (November – March, prevalence 4.76%) than in the wet seasons (April – October, prevalence 3.51%), this especially driven by high prevalences in the months of November (5.39%) and December (5.96%). This is similar to the findings of Nwanta et al. (2011) in Enugu State, Bikom et al. (2021) in Cross River State of Nigeria and Ndukum et al. (2010) in Cameroon. However, it is in contrast to the findings of Adamu et al. (2017) in Gombe and Akinbobola et al. (2017) in Abuja. The dry season is a period of drought with very little grass and water, among other challenges such as reductions in general performance of animals, increased susceptibility to diseases, migration of flocks and overcrowding of available grazing land (Lamidi and Ologbose, 2014). During these stressful periods of drought and varying temperature, animals that are sick and weak and may not be able to travel are sold and many of them are slaughtered in the abattoir. It is possible that weakness and wasting in these animals are signs of a patent TB infection, and this would explain why more of these animals are found in the abattoir during the dry season.

There was a direct relationship between age groups and prevalence of TB in cattle. Cattle within the older age groups had higher prevalences of TB (age 5-10 years, prevalence 6.53%, age >10 years, prevalence 8.11%) than those within the younger age group (age <5 years, prevalence 1.67%). This agrees with the findings of Sa'idu et al. (2015b) in Bauchi State, Sonfada and Garba (2000) in Sokoto State, Adang et al. (2015) in Gombe State, Cook et al. (1996) in Zambia and Inangolet et al. (2008) in Uganda. This result may be due to the chronic pattern of TB in cattle and older cattle being more likely to have had an exposure to TB than younger ones (Boschiroli and Thorel, 2010).

Gender was a significant risk factor for the prevalence of TB among cattle slaughtered at the abattoir. TB lesions were more likely to be seen in males than in females (adjusted odds ratio for females 0.35, $p < 0.001$). This is similar to the findings of Kazwala et al. (2001) who observed that in the southern highlands of Tanzania, castrated bulls are kept longer to improve their body size and increase their market price. Entire bulls with desirable traits are sometimes shared between different herders for breeding and this may expose them to diseases (Mgongo et al., 2014).

The risk of TB was significantly lower in the northern (AOR 0.06, CI 0.56 – 0.63, p= <0.001) and southern geopolitical zones (AOR 0.28, CI 0.23 – 0.33, p= <0.001) compared to the central zone. The reason for these differences was not clear, although it might in part be because the abattoir is located in the northern zone and due to the long distance, people from the southern zone find it difficult to bring TB affected cattle to the abattoir for slaughter.

Although higher prevalence of TB in the White Fulani breed and the lower prevalence of TB in the Muturu breed of cattle has been demonstrated in studies by Adang et al. (2015), Ahmad et al. (2017) and Jajere et al. (2018), breed did not appear to be a significant risk factor for TB in this study. The prevalence was highest in mixed breeds of cattle (4.39%) followed by the White Fulani breed (4.04%) and the lowest prevalence was seen among the Muturu breed of cattle (3.48%), but the differences were not significant ($\chi^2 = 5.46$, 3 d.f., p=0.141). The high prevalence of TB seen among the White Fulani breed may be due to its high susceptibility and low immunity to infection compared to other breeds (Kim et al., 2017; Tijjani et al., 2019). The low prevalence seen among the Muturu breeds of cattle maybe due to it being less prone to infection than other breed and are well adapted to local diseases and are thus rarely ill (Blench, 1999).

Humans

The overall prevalence of TB in humans in this study was 14.2% which is very similar to the 14.7% in a retrospective study of patients attending infectious diseases hospital, in Kano, Nigeria reported by Imam and Oyeyi (2010) and a little higher than the 12.9% reported by Nwanta et al. (2011) also in a retrospective study in Enugu, Nigeria. But the result was found to be low compared with the 32.5% reported by Chigbu and Iroegbu (2005) among patients attending a chest clinic in Aba, Eastern Nigeria and 21.6% by Nwachukwu and Peter (2010) in studies using direct sputum smear microscopy for TB diagnosis in Umuahia, Abia state, Nigeria. This variation of TB prevalence in these locations may be due to limited laboratory facilities, inadequate number of trained laboratory staff, inadequate access to power source and weak laboratory information system (Obasanya et al., 2015).

The significantly high prevalence of TB in humans in the dry season (16.3%) compared to the wet season (12.2%) agrees with the findings of Kusimo et al. (2015) in Nigeria but is in contrast with the findings of Desalu (2011) in Nigeria and Ane Anyangwe et al. (2006) in Cameroon. The dry season is characterised by dusty, dry harmattan which when inhaled can trigger reactions that can cause coughing. This might have triggered TB screening in humans as seen in the study where many people were tested for TB in the months November to March which falls in the dry season.

Even though there were more cases of TB in humans among males (n = 3,445) than among females (n = 3,264), gender was not a significant risk factor for TB in humans in Plateau State (univariable analysis p = 0.253). This is similar to the findings of Chamla et al. (2004) in China and Kpanyen et al. (2011) in Liberia in which there were more TB cases among males than females. Gender can affect TB exposure because of the differences in social roles and activities. Males may travel more frequently to work and spend more time in settings that may be

conducive for TB transmission like the markets, abattoir, and pubs (Nhamoyebonde and Leslie, 2014).

Age group was found to be a significant risk factor for TB in humans. The highest prevalence of TB in humans was seen among the age group 21-40 (17.93%) and the lowest was seen among persons more than 60 years old (6.30%), with corresponding adjusted odds ratios of 1.74 (CI 1.63 – 1.86, p<0.001) and 0.58 (CI 0.48 – 0.69, p < 0.001) respectively in relation to the reference age group of less than 20 years old. This is similar to the findings of Nwanta et al. (2011) in Enugu State and the study by Nwachukwu and Peter (2010) in Abia State where the prevalence of TB was highest in the age group 26-35 years and lowest among persons greater than 56 years old. The young adult is more vulnerable to infection because at this child-bearing age people are highly sexually active, hence at risk of exposure to HIV. This high degree of vulnerability of TB infection among young adults in Nigeria may be due to the high incidence of HIV/AIDS among this group, according to a report by the WHO (2017). There is a complex relationship between HIV and TB infection. HIV increases susceptibility to TB infection (Obionu, 2007). These results are alarming as these age group forms the workforce of the country and many of these people are the breadwinners of their families.

TB in humans was most prevalent in the population of persons from the northern zone of Plateau State (16.4%) and least among those from central zone of the state (12.9%). Adjusted odds ratios of 1.70 (CI 1.59 – 1.83, p < 0.001) and 1.44 (CI 1.35 – 1.54, p < 0.001) for the northern and southern geopolitical zones respectively signified increased risk of human TB in relation to the reference central zone, in contrast to the situation in cattle in which the northern and southern zones had lower risk of TB. This may be connected to the high prevalence of HIV (16.07%) in Jos North LGA of the state (Gomwalk et al., 2012).

There are some similarities in the annual prevalence of TB in humans and cattle over the years of the study. Overall, there was a broadly declining TB prevalence in both the human and cattle

populations based on both seasonal-trend decompositions by Loess and generalised linear models. The apparent trend seen in cattle may have resulted in part from increased disease surveillance in the Jos Abattoir in 2006 and 2007 resulting from efforts to control highly pathogenic avian influenza, and in part from decreased disease surveillance from 2012 onwards resulting from civil insecurity. The reason for the decline in prevalence of human TB was less clear but may have been due to increased awareness of diseases over the period. There are also some significant contributions to the already existing TB control services in Plateau State by the private sector which have yielded positive results (Ibrahim et al., 2014).

2.7 Conclusion and Recommendation

The findings from this study have documented the prevalence and risk factors for TB in cattle and humans in Plateau State using retrospective data. Although there is a decline in the prevalence of TB in both the human and cattle population, the high prevalence of TB seen suggests the disease is endemic in Plateau State. In view of this, the zoonotic TB appears to be potentially high. Control measures such as increasing the awareness of zoonotic TB among abattoir workers and the public and strengthening routine meat inspection of slaughter animals in the abattoir should be implemented.

2.8 Limitations

Generally, routine abattoir meat inspections are meant to provide useful information regarding the quality and health of the meat. However, this survey has a limitation because the data may have been underestimated because of imperfect diagnostic tools (which might have contributed to missing cases), incomplete information and poor record-keeping system as this is done manually and some records were misplaced. However, the degree of completeness of cattle dataset was 91.23% and human dataset was 100%.

The standard error derived from the cattle logistic regression model should be taken with caution due to the detection of serial correlation in the cattle TB monthly prevalence data.

CHAPTER THREE: *MYCOBACTERIUM TUBERCULOSIS* AS THE LEADING CAUSE OF TUBERCULOSIS IN SLAUGHTERED CATTLE IN PLATEAU STATE

3.1 Introduction

TB is a chronic and contagious disease of humans and many different animal species and the leading cause of death among people infected with HIV/AIDS with about 251,000 deaths worldwide in 2018 (WHO, 2019b). In cattle, *M. bovis* generally is the causative agent (Morris et al.,1994; de Lisle et al., 2001). *M. bovis*, has one of the widest host ranges of all pathogens (Morris et al.,1994; de Lisle et al., 2001). As it is zoonotic, bTB constitutes an important public health problem (Sa'idu et al., 2015a). *M. tuberculosis* is the primary cause of TB in humans (Smith et al., 2006a). Both TB pathogens can be transmitted from animals to humans and vice versa (Ameni et al., 2013; Frietsche et al., 2004; Michalak et al., 1998; Ocepek et al., 2005). Humans are the main host and reservoir for *M. tuberculosis* (Fitzgerald et al., 2015). *M. tuberculosis* has been reported sporadically in animals, especially those that have prolonged close contact with humans such as cattle (Adesokan et al., 2019; Alfonso et al., 2004; Montali et al., 2001; Pavlik et al., 2003; Woldemariam et al., 2020).

In Nigeria, little is known about the interface between people and livestock with regard to transmission of TB (Cadmus et al., 2018). Learning more about TB prevalence and type found in cattle could help in understanding the potential for transmission (Cadmus et al., 2018; Okeke et al., 2014).

In Nigeria, the diagnosis of TB in cattle is based on routine abattoir post-mortem examination of carcasses for lesions suggestive of TB. Biffa et al. (2010), in a study in Ethiopia, demonstrated that the routine post-mortem inspection in abattoirs failed to detect most disease cases as confirmed by laboratory diagnostic tests. A quick and accurate method for the detection of mycobacteria is important in the effective control of TB in both human and animal

populations. The bacteriological culturing of mycobacteria has been the 'gold standard' test for the diagnosis of TB (Cavalhiero et al., 2020). It has been shown to be time consuming (taking about 2-3 months), to have a low degree of sensitivity and a propensity for producing some false negative results especially during the early stages of the disease. Detection of AFB can only give a preliminary diagnosis of TB (Duffield et al., 1989; Saito, 1998). Amplification of specific sequences of a target DNA by the PCR technique provides more rapid confirmation and is especially useful for the rapid detection of fastidious or slowly growing pathogens (Cousins et al., 1991; Moser et al., 1989; Olive, 1989).

3.2 Aims of the study

1. To determine prevalence of TB based on detailed post-mortem examination of selected cattle slaughtered at the Jos abattoir, Plateau State
2. To isolate and identify mycobacteria species responsible for causing TB in cattle slaughtered at the Jos abattoir, Plateau State using Real-time PCR

3.3 Study site

The study was conducted at the Jos abattoir located in Jos south local government area (L.G.A) in the northern geopolitical zone of Plateau State. The Jos abattoir is the major source of meat and other animal products in the state, and it is under the control of the Plateau State Ministry of Agriculture. About 35-40 cattle/day, 50-100 sheep and goats/day, 4-6 pigs/day and 10 camels/week are slaughtered in the abattoir (Yilzem et al., 2017). The geographical coordinates of the abattoir are longitude 9^{o} 54 North and Latitude 8^{o} 53 East.

3.4 Materials and methods

3.4.1 Study design

A cross-sectional study with systematic random sampling was carried out to select and investigate the carcasses of slaughtered cattle in Jos abattoir in Plateau State from February to

April 2017. About 40 cattle were slaughtered daily and every 5th animal slaughtered was chosen and supervised. Tissue samples of lesions suggestive of TB were collected from cattle slaughtered at the abattoir for DNA extraction and molecular characterization. Data on the animals sampled, including age, gender, body condition, breed and location of settlement were documented.

3.4.2 Sample size

All cattle slaughtered at the abattoir during the study period were considered for sampling. The calculation of sample size was based on 50% prevalence, 5% desired absolute precision and 95% confidence level (Thrusfield, 2005).

$$n = \frac{z^2 \times p(1-p)}{d^2}$$

Where n = required sample

P = prevalence (0.5)

d = desired absolute precision (0.05)

Z = normal distribution constant/ value for the corresponding Confidence level (1.96 for 95% confidence)

Desired absolute Precision (Margin of error) $z = \frac{\sqrt{\rho(1-\rho)}}{n}$

The sample size calculated was 384. The precision was increased by 20% and a total number of 500 animals were selected for the study. The desired absolute precision was calculated to be 0.043. Hence there was a 95% confidence that the true value is within +/- 0.043.

3.4.3 Study variables

The independent variables used in this study were age group, gender, body condition, breed and location. Age was categorised into three age groups: <5, 5-10 and >10 years; Gender was categorised as female and male. In relation to body condition, three categories were

constructed: good, moderate, and poor according to Nicholson and Butterworth (1986) (Table 3.1). Breed were categorised as White Fulani, Red Bororo, Muturu and mixed breed of cattle. Location of settlement of cattle was categorised as northern, central, and southern geopolitical zones. The dependant or outcome variables was TB status (TB case/non-TB case).

3.4.4 Post-mortem examination and sample collection

I carried out a detailed post-mortem examination of each animal selected according to the Manual on meat inspection for developing countries by Herenda et al. (2000). A total of 500 cattle was selected and examined. The age, gender, breed, and location of settlement of cattle was recorded for all slaughtered cattle, achieving data completeness of 100%. The age of the cattle was determined by asking the cattle owner and the body condition was recorded as good, moderate, or poor (Figure 3.1, 3.2, 3.3). Examination was done by inspection, palpation and incision of carcass and tissues after slaughter. Cases were based on gross detection of typical tubercle, nodular lungs, gritty calcification on incision and yellow granulomatous lesions in tissues. The lungs were sliced at 2cm intervals, and each slice was inspected and palpated. If a suspected tuberculous lesion was present, tissue samples were collected in sterile universal containers with screwed caps (Figure 3.4). The samples were transported in an ice box with packed ice to the biosecurity laboratory level 3 (BSL3) of the National Veterinary Research institute (NVRI), Vom, Plateau State, Nigeria for storage at -20ºC and DNA extraction. Multiplex Real-Time PCR assay was performed in the diagnostic laboratory of the Langford Veterinary School, University of Bristol, United Kingdom.

Also, information was obtained on the number of cattle slaughtered and number of those with lesions suggestive of TB during routine post-mortem examination by abattoir meat inspection staff at the time of this study.

Figure 3. 1 Photograph of cattle with good body condition at the Jos abattoir. 2017

Figure 3. 2 Photograph of cattle with moderate body condition at the Jos abattoir. 2017

Figure 3. 3 Photograph of cattle with poor body condition at the Jos abattoir. 2017

Table 3. 1 Body condition of cattle and the description

Body Condition	Description
Poor	The animal's bony structures are prominent and clearly defined. Low level of musculature of fat
Moderate	The animal has a fair degree of muscle and fat tissue. There is no prominent backbone nor ribs. The pins are filled out but not mounded. The tubal coxa ('hip') remains prominent.
Good	The animal is smooth and blocky in appearance. It has obvious substantial levels of fat and muscle tissue covering, especially around the pins and flanks. Most skeletal definition difficult to identify.

(Nicholson and Butterworth, 1986)

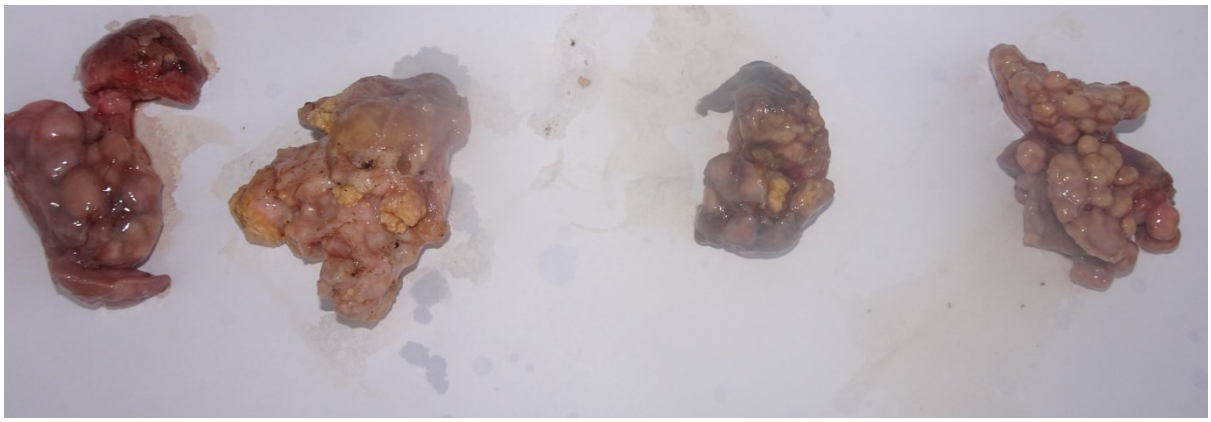

Figure 3. 4 Photograph of suspected TB infected lung tissues collected from the Jos abattoir showing tubercules and caseous necrosis. 2017

3.4.5 DNA extraction

DNA was extracted using Qiagen DNA extraction kit in accordance with the manufacturer's instructions. Samples stored at -20^{O}C were allowed to thaw at room temperature and approximately 25mg of tissue was homogenized with a mortar and pestle containing bead mill and 5ml of lysis buffer. 20µl of proteinase K was added, vortexed and incubated at 56 ^{O}C until the tissues were completely lysed. The lysate was vortexed and 200µl ethanol (100%) was added and vortexed. This mixture was then pipetted into a DNeasy Mini spin column and placed in a 2ml collection tube and centrifuged at 8000rpm for 1 min. The flow-through and collection tube was discarded, and the DNeasy Mini spin column was then placed in another 2ml collection tube where 500µl pre-wash buffer was added and centrifuged for 3 mins at 14000rpm to dry the DNeasy membrane. Again, the flow-through and collection tube was discarded. The DNeasy Mini spin columns were placed in a clean 2mls centrifuge tube and 200µl wash Buffer was added directly onto the DNeasy membrane to elute DNA and then incubated for 1 min and centrifuged for 1 min at 8000rpm. The eluted DNA was stored at -20^{O}C until needed for molecular analysis.

3.4.6 Multiplex real-time PCR assay

Multiplex real-time PCR enables the simultaneous detection of several target sequences by incorporation of multiple sets of primers. This was performed according to the specification of Pinsky and Banaei, 2008. The primers used for this study are listed in table 3.2. A total reaction volume of 25µl was used for each sample. This was made up of 2.5µl of each primer, 1.0µl of sybr green, 12.5µl of Gotaq universal mastermix and 6.5µl of extracted DNA. Water and known *M. bovis* strain was used as negative and positive control, respectively.

Reaction 1 included Genus control Forward and Genus control Reverse primers to a region of 16SrRNA gene that is common in all mycobacteria. Reaction 2 included primers (RD9 Present

Forward and RD9 Present Reverse) to detect the presence of RD9. Reaction 3 included primers (RD4 Common Forward and RD4 Present Reverse) to detect the presence RD4. Reaction 4 included primers (RD4 Common Forward and RD4 Deleted Reverse) to detect the absence of RD4. Reaction 5 included primers (RD1 Deleted Forward and RD1 Deleted Reverse) to detect the absence of RD1 (Table 3.2).

In this manner, a distinction can be made between various species within the MTC, according to a schedule by Pinsky and Banaei (2008). This allows for the definitive identification of *M. tuberculosis*, other mycobacteria specie (*M. africanum, M.caprae, M. pinnipedii, M. microti*), *M. bovis* and *M. bovis* BCG respectively. The evolutionary scenario for this is presented in figure 3.5.

Table 3. 2 Primers and product characteristics used in this study (Real-Time PCR Assay)

Description of target	Product length (bp)	[a]Product T_m(°C)	Primar name	Primer sequences (ref: Pinsky BA and Banaei N, 2008)
RD9 present	51	76.3 ± 0.1	RD9 Present Forward	TTTCGAGCCGTAAATTACTGTG
			RD9 Present Reverse	GAGCATTCTCGCTCCGAAT
RD1 deleted	226	86.2 ± 0.6	RD1 Deleted Forward	GGATTTGACGTCGTGCTTCT
			RD1 Deleted Reverse	TTCAACGGGTTACTGCGAAT
RD4 present	55	77.8 ± 0.2	RD4 Common Forward	AGAAGCGCAACACTCTTGGA
			RD4 Present Reverse	CATGCGCCCTATTTGATCTC
RD4 deleted	94	83.0 ± 0.1	RD4 Common Forward	AGAAGCGCAACACTCTTGGA
			RD4 Deleted Reverse	TTGCTGAAAAATGGCTATTGA
Mycobacterial 16S Rrna	78	79.3 ± 0.1	Genus Control Forward	CAACGCGAAGAACCTTACCT
			Genus Control Reverse	TGCACACAGGCCACAAGGGA

[a] the product melting temperatures (T_m) values represent means ± 95% confidence intervals

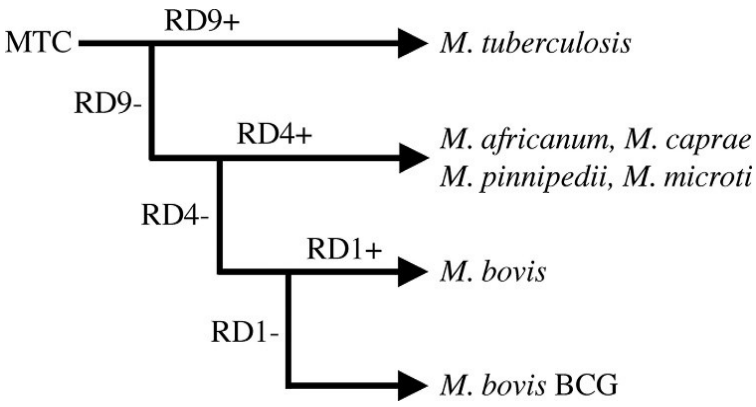

Figure 3. 5 An evolutionary scenario for MTC which shows the presence or absence of regional difference (RD) RD9, RD4 and RD1

Source: Pinsky and Banaei (2008)

3.4.7 Data analysis

Data obtained were entered into MS Excel 2010 (version 1.0) and analysed with Stata/MP version16.0 statistical software. The characteristic of selected cattle was summarised for all categorical variables and expressed as simple descriptive frequency and percentages. The age of cattle was summarised with median, range and interquartile range (IQR). TB cases of slaughtered cattle according to their age group, gender, body condition, breed and location, and breed was compared using chi-square (χ^2) tests of association.

For cattle that were chosen to be examined in detailed, TB prevalence was calculated for all variables as the total number of cattle with lesions suspected to be TB divided by the total number of cattle slaughtered. The overall total prevalence was calculated as the total number of TB cases of cattle with lesions suspected to be TB divided by the total number of cattle slaughtered. Prevalence was presented in percentages with their confidence interval. $P < 0.05$ was considered significant for this study.

For cattle examined routinely, the overall total TB prevalence was calculated as the total number of TB cases of cattle with lesions suspected to be TB divided by the total number of cattle slaughtered over the period of study and presented in percentages.

3.4.8 Permission

Permission to conduct the study in Jos abattoir was granted by the Plateau State Ministry of Agriculture.

3.5 Results

3.5.1 Characteristics of cattle

A total of 500 cattle slaughtered at the abattoir were examined within a period of 9 weeks. The median age of the cattle examined was 7 years (Range = 15, IQR = 6). Most of the cattle examined were within the age groups 5-10 years old (n = 268, 53.60%), males (n = 388, 77.60%), had good body condition (n = 237, 47.40%), were White Fulani breed (n = 360, 72.00%) and were from the central geopolitical zone of Plateau State (n = 292, 58.40%) (Table 3.3).

Table 3. 3 Characteristics of slaughtered cattle supervised at the abattoir (N = 500)

Variables		Frequency	Proportion of total (%)
Median age = 7yrs (Range = 15, IQR = 6)			
Age group			
	<5 years	143	28.60
	5-10 years	268	53.60
	>10 years	89	17.80
Gender			
	Female	112	22.40
	Male	388	77.60
Body condition			
	Good	237	47.40
	Moderate	139	27.80
	Poor	124	24.80
Breed			
	White Fulani	360	72.00
	Red Bororo	102	20.40
	Muturu	15	3.00
	Mixed breed	23	4.60
Location (geopolitical zone)	Northern	164	32.80
	Central	292	58.40
	Southern	44	8.80

3.5.2 Chi square test and prevalence of TB in slaughtered cattle examined in Jos abattoir

Out of the 500 slaughtered cattle examined, lesions suggestive of TB were seen in 98 of the cattle giving an overall TB prevalence of 19.60% (Table 3.4).

Slaughtered cattle within the age group less than 5 years old had the lowest number of TB case (n=13) and cattle within age group 5-10 years old had the highest number of TB cases (n=53). The number of TB cases varied across the age groups of cattle (χ^2=25.1344, P= <0.001). The prevalence of TB was highest in cattle within the age group more than 10 years old (35.96%, CI=26.67-46.42) and the least TB prevalence was seen in cattle within the age group less than 5 years old (9.09%, CI=5.34-15.42) (Table 3.4).

The lowest number of TB cases was seen among female cattle (n=32) and the maximum number of TB case was seen among male cattle (n= 82). The number of TB case did not vary across gender of slaughtered cattle (χ^2=2.5866, P= 0.137). TB was shown to be more prevalent among male (21.13%, CI=17.35-25.46) than female cattle (14.29%, CI=8.94-22.08) (Table 3.4).

The lowest number of TB case was seen among cattle that had good body condition (n=25) and highest among those that had poor body condition (n=42). TB case varied between the different body conditions of the cattle (χ^2= 28.9916, P= <0.001). The prevalence of TB in cattle was highest among cattle with poor body condition (33.87%, CI=26.08-42.65) followed by those that had moderate (22.30%, CI=16.13-29.99) and the lowest TB prevalence was seen among those with good body condition (10.54%, CI=7.22-16.16) (Table 3.4).

The lowest number of TB cases was found among the mix breed (n=1) and the highest found among the White Fulani breed of cattle (n=67). The number of TB case varied across the breed of cattle (χ^2=8.5794, P= 0.032). The lowest TB prevalence was seen among the mixed breed

(4.35%, CI=0.61-25.31) and the highest was among the Muturu breed of cattle (40%, CI=19.14-65.25) (Table 3.4).

The number of TB case was shown to be lowest among cattle from the southern zone (n=6) and highest among cattle from the central zone (n=69). The number of TB case varied across the three geopolitical zones of Plateau State (χ^2= 7.2380, P= 0.028). TB was shown to be most prevalent in cattle from the central zone of the state (23.63%, CI=19.10-28.89) followed by those from northern zone (14.02%, CI=9.49-20.24) and the lowest was seen among those from the southern zone of the state (13.64%, CI=6.24-27.24) (Table 3.4).

Table 3. 4 Prevalence (95% confidence intervals) of TB in selected cattle and Chi square test of association between TB case and age group, gender, body condition, breed, and geopolitical zone (location) of slaughtered cattle at Jos abattoir

Variables		**No. of cattle examined**	***TB case**	**Prevalence (%)**	**95% Confidence Interval**		**Chi-square (x^2)**	**P-value**
Age group							25.1344	<0.001
	<5 years	143	13	9.09	5.34	15.42		
	5-10 years	268	53	19.78	15.42	24.99		
	>10 years	89	32	35.96	26.67	46.42		
Gender							2.5866	0.137
	Female	89	32	14.29	8.93	22.08		
	Male	388	82	21.13	17.35	25.49		
Body condition							28.9916	<0.001
	Good	237	25	10.55	7.22	16.16		
	Moderate	139	31	22.30	16.13	29.99		
	Poor	124	42	33.87	26.08	42.65		
Breed							8.5794	0.032
	White Fulani	360	67	18.61	14.91	22.98		
	Red Bororo	102	24	23.53	16.28	32.74		
	Muturu	15	6	40.00	19.14	65.25		
	Mixed breed	23	1	4.35	0.61	25.31		
Location (geopolitical zones)							7.238	0.028
	Northern	164	23	14.02	9.49	20.24		
	Central	292	69	23.63	19.1	28.89		
	Southern	44	6	13.64	6.24	27.24		
Total		**500**	**98**	**19.6**				

*No. with lesions suggestive of TB

P<0.05 significant level

3.5.3 Overall prevalence of TB in slaughtered cattle routinely examined and those examined in detail at Jos abattoir

During the time of this study, a total of 1,640 cattle carcases were routinely examined by abattoir staff, out of which 66 cattle were found to have lesions suggestive of TB and this gave a prevalence of 4.02%. We examined a total of 500 cattle carcasses in detail and found 98 cattle with lesions suggestive of TB resulting in a prevalence of 19.6% (Table 3.5). Figure 3.7 shows photograph taken at the abattoir of normal and TB infected lungs from cattle slaughtered at the abattoir.

Table 3. 5 Summary of cattle supervised in the abattoir during period of study

	No. of cattle supervised	No. of TB cases	Prevalence (%)
Routine meat examination by abattoir staff	1,640	66	4.02
Detailed meat examination by researcher	500	98	19.6
Total	**2,140**	**140**	**6.54**

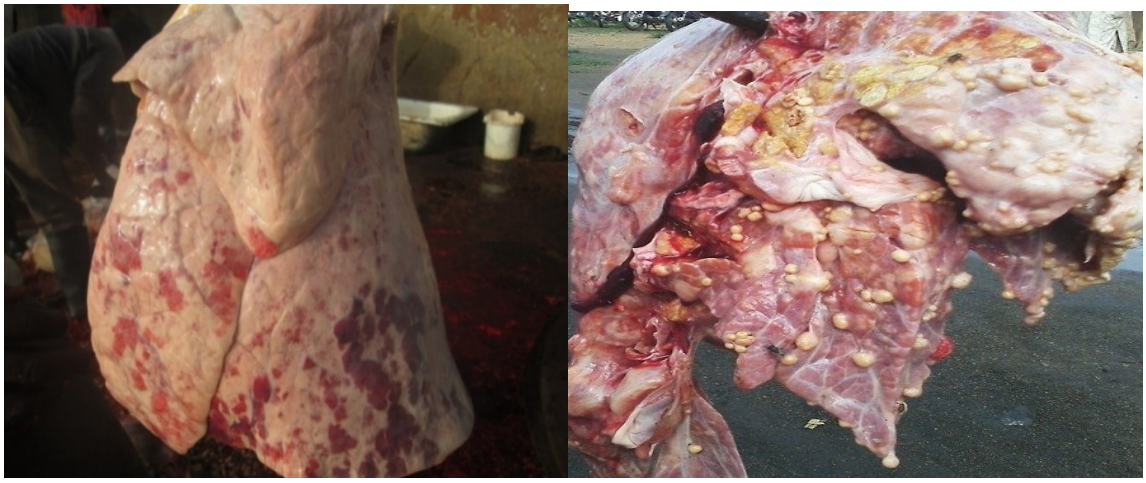

Figure 3. 6 Photograph of normal and TB infected lungs detected during routine meat inspection at the Jos abattoir. 2017

3.5.4 Molecular characterization of mycobacteria using real-time PCR

Table 3.6 shows results of the genomic deletion analysis of tubercle bacilli strain. Out of 98 samples from 98 slaughtered cattle with lesions suggestive of TB, MTC could be identified in 88 animals. Further differentiation shows that, of the 88 MTC samples, 79 samples were *M. tuberculosis*, two were other mycobacterium (*M. africanum, M. caprae, M. pinnipedii, M. microti*). There was no *M. bovis* nor *M. bovis* BCG identified and 7 samples found to contain unidentified mycobacteria.

Table 3. 6 Genomic deletion analysis of tubercle bacilli specie isolated from 98 slaughtered cattle in the abattoir using real-time PCR

Reactions	Region of difference and clonal complex	Present	Absent	Types of mycobacteria	Concordance
1	16SrRNA gene. n=98	88	10	MTC	89.79%
2	RD9 n=88	79	9	*M. tuberculosis*	89.77%
3	RD4 presence n=9	2	7	*M. africanum, M. caprae, M. pinnipedii, M. microti*	2.27%
4	RD4 absent n =7	0	7	*M. bovis*	0.0%
5	RD1 n=7	0	7	*M. bovis BCG*	0.0%
	Unidentified	7		Unidentified mycobacterium	7.95%

n= number of isolates= number of cattle

3.6 Discussion

TB is a disease with potential public health and economic importance and because of the increasing contact between cattle and humans due to the husbandry practices in Plateau State, it was necessary to review the current prevalence of TB in cattle and characterize the disease-causing pathogen to aid in infection control and spread of zoonotic and reverse zoonotic TB in the state.

Post-mortem

In the current study, the prevalence of TB increased with the age group of the cattle. This is in line with other studies in some states in Nigeria by Ibrahim et al. (2012) in Jigawa State, Usman (2016) in Gombe State, Saidu et al. (2015) in Bauchi State, Cadmus et al. (2004) in Ibadan and in other African countries including studies by Ameni et al. (2007), Gumi et al. (2011), Fetene and Kebede (2009) in Ethiopia; Katale et al. (2013) in Tanzania, Nalapa et al. (2017), Munyeme et al. (2008) in Zambia and Egbe et al. (2016) in Cameroon. This is likely due to older cattle being exposed to the agent of TB over a longer period. This is in contrast with a study in Northern Ireland by Byrne et al. (2017) in which there was a decline of TB prevalence in cattle above 36 months old due to the early detection and removal of infected animals. Results also showed that TB prevalence was highest among cattle that had poor body condition. Poor body condition may be due to malnutrition. This could weaken the animals' immune system and hence they may be more susceptible to infection with TB and/or other diseases. Poor body condition is however also a clinical sign that follows an active infection with the TB agent in cattle. According to a study by Kazwala et al. (2001), as a result of a progressive long-lasting pathological process, animals with a clinically advanced TB infection often present with poor body condition.

The result on routine post-mortem inspection of slaughtered cattle shows that 66 out of 1,640 slaughtered cattle were identified with lesions suggestive of TB giving a prevalence of 4.02%, while result of detailed meat inspection revealed that 98 out of 500 slaughtered cattle were found with TB lesions giving a prevalence of 19.6% (Table 3.5). This indicates that there is underestimation of the occurrence of TB in cattle in Plateau State. This may be due to there being just a few localised lesions which might have been missed, limited time spent on examining organs during routine post-mortem inspection, limited experience and training of abattoir workforce, and lack of standardization of meat inspection protocol in the abattoir (Biffa et al., 2010). This study has demonstrated that with detailed meat inspection, more cases of TB in cattle can be detected and hence a more accurate estimate of the prevalence of TB found. Detailed abattoir meat inspection has proven to be a satisfactory procedure for the detection of TB in slaughtered cattle. This is in accordance with a study by Biffa et al. (2010) who evaluated detailed and routine meat inspection protocols for the detection of lesions in slaughtered cattle and reported that a detailed meat inspection protocol showed higher sensitivity than routine meat inspection protocol which failed to detect the majority of infected carcasses confirmed by culture and macroscopy.

Molecular characterization

In this study, *M. tuberculosis* which is a natural pathogen of humans was identified in cattle in Plateau State. *M. tuberculosis* is zoonotic, and some strains are known to be highly virulent. As such, *M. tuberculosis* infection in cattle is a public health concern because there is a potential risk of it spilling back to humans, more especially to those with compromised immune systems. This situation is known to occur mostly in countries with high incidence of TB in humans (Romero et al., 2011). Studies have been conducted in Africa and Asia that have also isolated *M. tuberculosis* in cattle. They include Ameni et al. (2011), Stefan et al. (2009) in Ethiopia; Boulahbal et al. (1978) in Algeria and Sulieman and Hamid (2002) in Sudan.

Although there is limited information about prevalence of *M. tuberculosis* in its spill over hosts, majority of studies on TB in cattle due to *M. tuberculosis* have reported a prevalence of ≤1% (Lesslie and Birn, 1970; Pavlik et al., 2003; Schliesser, 1976; Steele, 1980; Thoen et al., 1981). However, individual studies conducted in Algeria, Sudan and Ethiopia reported a higher prevalence of *M. tuberculosis* infection in cattle to be 6.2%, 7.4% and 27%, respectively (Ameni et al., 2011; Boulahbal et al., 1978; Sulieman and Hamid, 2002). This may probably be due to the high prevalence of TB in humans in these countries (Abdallah and Ali, 2012; Amrane, 1996; Sharaf Eldin et al., 2011; WHO, 2019b). Humans with active TB are believed to be the main source of transmission of *M. tuberculosis* to animals which usually occurs via aerosol (Ibrahim et al., 2016; Thoen et al., 1981). There are studies that were able to trace the source of *M. tuberculosis* infection in cattle to humans with active TB using molecular typing techniques to compare and establish the epidemiological relatedness of the strains from both cattle and humans (Ameni et al., 2011; Ocepek et al., 2005; Romero et al., 2011; Spicic et al., 2012).

In this study, real-time PCR was used to differentiate MTC. Real-time PCR is more rapid, sensitive, and specific in detecting MTC than the conventional PCR which is laborious and prone to errors and contamination due to post amplification handling. The advantages of real-time PCR assay include the short time needed for conducting the test, the decreased risk of contamination due to automation and its ability to quantify the bacteria load in the sample (Agamy et al., 2017; Katoch, 2004b; Kaur and Singh, 2016; Sethi et al., 2012). The results obtained were overwhelming as out of 88 cattle infected with mycobacteria, 79 (89.77%) were found to be infected with *M. tuberculosis* and none of the cattle were infected with *M. bovis* (Table 3.6). This is in contrast to previous theories that cattle are resistant to infection by *M. tuberculosis* (Biet et al., 2005). These findings may have been due to transmission from infected humans, especially pastoralists or herders as they are known to have close and

prolonged contact with cattle (Alemayehu et al., 2008; Cadmus et al., 2009; Munyeme et al., 2009; Pavlik et al., 2003; Thoen et al., 2009).

In Nigeria, Adesokan et al. (2019) isolated *M. tuberculosis* Uganda I strain from milk samples from cattle, Jenkins et al. (2011) isolated *M. tuberculosis* from tissue samples from cattle slaughtered at the abattoir, Cadmus et al. (2008) isolated *M. tuberculosis* from milk samples and tissue samples from cattle slaughtered at the abattoir. There are also studies that have isolated *M. tuberculosis* and *M. bovis* from livestock workers in Nigeria (Adesokan et al., 2012; Cadmus et al., 2018). Therefore, a possible interface between bTB and human TB could be implied in Nigeria. Also, the high number of *M. tuberculosis* infection in cattle in this study indicates a potential for cattle-to-cattle transmission of *M. tuberculosis.* Hence, there could be a possibility of cattle becoming a reservoir host of *M. tuberculosis*. Although this need to be further investigated using genotyping to gain a better understanding of the epidemiology of *M. tuberculosis* infection in cattle and their potential to sustain infection in the absence of human reservoir.

Although *M. tuberculosis* infections in humans in Nigeria is endemic, the prevalence in cattle has not been known before. This study's findings provide arguments for further investigation of incidence of TB in cattle due to *M. tuberculosis* in Plateau State. The high number of potential human mycobacteria pathogens detected among cattle of economic importance have grave implications for any strategies adopted for the prevention and control of TB in humans and cattle.

3.7 Conclusion

The high prevalence of TB in cattle indicates that the disease is endemic and that there is a high infection rate prevailing in the general population of cattle in Plateau State and most likely in the country at large.

There is widespread underreporting of TB in abattoirs and zoonotic transmission of TB appears to be potentially high as infected meat may have been passed as fit for human consumption. This indicates that routine abattoir inspection needs to improve.

There is some evidence of human-to-cattle transmission of TB and possible circulating human strain in cattle population, which might have additional zoonotic risk to humans in close contact with animals (although we have not directly got evidence for this).

The real-time PCR assay contributes significantly to identifying isolates of MTC to species level which is important for understanding the epidemiology of the disease and can aid in planning control measures and in optimizing treatment.

3.8 Recommendation

Standardisation of meat inspection protocol, training of meat inspection personnel to refine their skills for detailed post-mortem examination and raising community public awareness of the disease are recommended as interventions to improve meat inspection service in Plateau State, Nigeria with an overall aim of protecting consumers from zoonotic TB.

Collaborative efforts between the veterinary and public health sectors of Plateau State in a One Health fashion should be encouraged to ensure that epidemiological investigations and reporting of TB infections are carried out in both the human and cattle populations in the state.

Clinical laboratories should be strengthened and supplied with current equipment so that they can carry out routine testing with the use of molecular techniques to identify and differentiate *M. bovis* from *M. tuberculosis* in Plateau State.

There is a need for policy creation to ensure mandatory routine TB screening of livestock personnel and cattle to control the transmission of TB at the human-animal interface.

Further investigations to identify humans as a source of *M. tuberculosis* in cattle should be carried out. The disease status of humans, especially that of livestock personnel who are in close contact with cattle should be investigated in Plateau State.

3.9 Limitation

Spoligo-typing and Variable Number of Tandem repeats (VNTR) was not carried out in this study to further identify the strain of *M. tuberculosis* circulating and compare it with existing data. This would have provided information on the prevailing strain among cattle in Plateau State and Nigeria at large and would have aided in the tracing of infection back to its root and aided in controlling it by quarantining and treating infected cattle.

Livestock personnel such as the abattoir workers, pastoralists, veterinarians, and other animal health workers were not screened for TB in the current study. This would have revealed evidence of direct links to possible ongoing reverse zoonosis of *M. tuberculosis* between the livestock personnel and cattle. These steps were beyond the immediate scope of the present study and will require further research.

CHAPTER FOUR: KNOWLEDGE ON BOVINE TUBERCULOSIS AND THE ZOONOTIC TUBERCULOSIS PREVENTIVE PRACTICES DEMONSTRATED BY LIVESTOCK WORKERS (FULANI PASTORALIST AND ABATTOIR WORKERS) AND THEIR ASSOCIATED DETERMINANTS

4.1 Introduction

TB in humans is caused mainly by the pathogen *M. tuberculosis* which is responsible for millions of TB infections in humans and hundreds of thousands of deaths globally (WHO, 2019b). Cattle is the major reservoir of *M. bovis* which causes bTB. It can also cause TB in humans referred to as zoonotic TB infection (Gompo et al., 2020). It is most often transmitted from animals to humans by the ingestion of infected milk, meat and by aerosol (Abubakar et al., 2011a; Ayele et al., 2004; Cadmus et al., 2006). Persons who handle infected herds such as the Fulani herdsmen, veterinary surgeons, animal farmers and abattoir workers are at risk of contracting zoonotic TB although only limited studies have investigated possible zoonotic TB infection in these groups of workers. (Adesokan et al., 2018; Ayele et al., 2004; Fekadu et al., 2018).

Currently, in developing nations, zoonotic TB is highly contagious due to livestock and humans living in the same premises and microenvironment, especially in rural areas. Another factor is the failure to implement control measures like pasteurisation of milk and the abattoir meat inspection is largely absent or inadequately carried out (Abubakar, 2007; Ayele et al., 2004; Cosivi et al., 1998a). In Africa, the incidence of TB in humans due to *M. bovis* has not been easy to detect due to technical challenges involved in the isolation of the microbe (Collins and Grange, 1983). The current prevalence rate of bTB at the national level is unknown although the organism, *M. bovis* has been isolated from unpasteurised raw milk and “nunu”(a home-made fermented sour milk produced by the introduction of starter culture into raw unpasteurised unskimmed milk), infected organs such as lungs, liver, lymph nodes, and heart

in abattoir tissues and from sputum and tissue biopsy samples from humans (Cadmus et al., 2006). Abattoir legislations are in place in Nigeria. They were established to ensure proper management of abattoirs, slaughter and processing of livestock for human consumption so that human health is protected (Abattoir Law, 2007). Unfortunately, these laws are not enforced.

In Nigeria, a major obstacle to the control and management of TB is the poor knowledge of the zoonotic transmission of TB (Fatiregun and Ejeckam, 2010).

The 2030 End TB strategy calls for an 80% reduction in the TB incidence rate by 2030 compared with levels in 2015 (WHO/OIE/FAO/IUATLD, 2017). In line with this, the government of Nigeria has put in place various TB intervention programmes aimed at early detection and treatment to control and ultimately to stop the spread of the disease in the country (Ogbuabor and Onwujekwe, 2019). Although these interventions contribute greatly to the management of TB, there are studies conducted in Nigeria and other countries that have pointed out some social challenges that could limit the effectiveness of any TB control programme. These include poor understanding of preventive practices, and of the cause, transmission, signs of TB and of some risky practices undertaken by people that may predispose them to the disease (Akeju et al., 2017; Alobu et al., 2014; Kigozi et al., 2017). Analysing the bTB knowledge and the zoonotic TB transmission predisposing practices of people in the livestock industry will provide useful insights for planning future TB control intervention in Plateau State and Nigeria more generally. It will also help to ensure a successful TB control plan and ultimately achieve the goal of 2030 End-TB strategy in Nigeria.

4.2 Aims of the Study

This study aims at investigating the knowledge on bTB and the preventive practices for zoonotic TB transmission and associated determinants amongst abattoir workers and Fulani pastoralist in Plateau State, Nigeria.

4.3 Methods

4.3.1 Study site

The study was conducted at the Jos abattoir and Kara cattle market in Plateau State, Nigeria.

Plateau State has a population of over 3 million people, making about 2.82% of the whole population in Nigeria (National Population Commission, 2006). The Jos abattoir is located within Jos South L.G.A. of Plateau State between longitude 80 48º W and latitude 90 94 º N in the north central geopolitical zone of Nigeria. The area is about 1,250 metres above sea level. The abattoir is under the control of the Veterinary Department of the Ministry of Agriculture and Natural Resources, Plateau State and licensed by the Federal Livestock Department and Pest Control Services, Federal Ministry of Agriculture and Rural Development.

Kara livestock market is also located in Jos South L.G.A. of Plateau State. The livestock market is solely for the sale of cattle and has a land area of about 450m x 450m^2 (Okeke-Agulu and Ochelle, 2019).

4.3.2 Study design and data collection

A cross sectional study was conducted among abattoir workers and Fulani pastoralist (herdsmen) using purposive sampling method. With the use of an interpreter, the aim of the project was explained, verbal consent was obtained for their participation in the survey and participation was voluntary. Data was collected through face-to-face interview using a structured questionnaire. A pilot survey was conducted on 5 abattoir workers and 5 Fulani pastoralists. The questionnaire was designed specifically for this study to assess knowledge on

bTB and zoonotic TB preventive practices demonstrated by abattoir workers and Fulani pastoralists. The questionnaire consisted of questions intended to obtain information about respondent's socio-demographic characteristics, knowledge on bTB and the zoonotic TB preventive practices demonstrated by abattoir workers and Fulani pastoralists. The first section consisted of the details of individual characteristics which is categorised into groups, and this included occupation type (abattoir workers, Fulani pastoralist); age group (<30, 30-40 and >40 years old), gender (female, male), and level of education (none, primary, and post-primary education). The second section consisted of knowledge on bTB which include awareness of bTB, knowledge of cattle-to-human TB transmission, knowledge of human-to-cattle TB transmission knowledge of modes of TB transmission and knowledge on the signs of TB in cattle. Awareness of bTB was defined as the participant ever hearing or recognising the name of the disease. If participant were not aware of bTB no more questions were asked relating to the disease. The third section focused on zoonotic TB preventive practices of participants which include: using personal protective equipment (PPE) at work, not cohabiting with cattle, not using same watering point as cattle, not consuming meat with lesions nor unpasteurized raw milk nor "nunu" and vaccination with BCG vaccine.

4.3.3 Study population

4.3.3.1 Fulani pastoralists

The Fulani are an ethnic group and are the major pastoral group in Nigeria with a population of approximately 15.3 million and there are about 300,000 Fulani pastoralist in Plateau State attracted by low tsetse vector and abundant of grass land in the state (Blench, 2004; Majekodunmi et al., 2013). Traditionally they are known to be nomadic herdsmen or pastoralist whose primary occupation is raising livestock and would travel long distances in search of grazing land for their herds. The Fulani pastoralist are the major source of meat and milk in Nigeria which is their major source of income (Majekodunmi et al., 2014). Due to the nature

of their livelihood, they are disadvantaged in their access to basic social amenities like healthcare, formal education, potable water, and electricity.

All Fulani pastoralists visiting the Kara cattle market and willing to be interviewed from 1st to 30th June 2016 were selected to participate in the study. A total of 384 Fulani pastoralists were invited to participate but only 207 of them were present and were interviewed, achieving a 53.9% response rate.

4.3.3.2 Abattoir workers

Abattoir workers are responsible for the process of slaughtering livestock and preparing the meat for sale. A total of 405 people work at the Jos abattoir which include meat inspection personnel, butchers, meat handlers, labourers, and cleaners. These workers are exposed to biological agents primarily through direct contact with infected animals, their blood or body fluids or their tissues (Johnson and Etokidem, 2019). All abattoir workers available at the abattoir and willing to be interviewed from 7th to 29th May 2016 were selected to participate in the study. One hundred and ninety-eight (198) abattoir workers were invited to be interviewed and all the respondents showed up and were interviewed achieving a 100% response rate.

4.3.4 Scoring of knowledge

The scoring of knowledge was based on whether a respondent expressed awareness of bTB, had knowledge of cattle-to-human TB transmission, knowledge of human-to-cattle TB transmission, knowledge of at least two modes of TB transmission and knowledge of signs of TB in cattle. A score of 1 for each “yes” and 0 for each “no” answer was allocated, and an overall score of knowledge was computed for each participant up to a maximum of five. On a scale of 0 to 5, participants that scored less than three were classified as not knowledgeable on bTB (<3 = not knowledgeable on bTB) and participant that scored a total of three or more were

classified as knowledgeable on bTB (≥ 3 = knowledgeable on bTB) which is equivalent to 60% or more.

4.3.5 Scoring of zoonotic TB preventive practice

The scoring of practice was based on whether a respondent admitted to not cohabiting with cattle, not using the same watering point as cattle, not consuming meat with lesions nor unpasteurised raw milk nor "nunu", using PPE to handle livestock and their products, and being vaccinated with BCG. Each "yes" answer attracted a score of 1 and each "no" answer attracted a score of 0. A total score for each participant was computed up to a maximum of five. Participants that scored a total of less than three were classified as demonstrating poor zoonotic TB preventive practice (<3 = Poor zoonotic TB preventive practice) while those that scored a total of three or more were classified as demonstrating good zoonotic TB preventive practice (≥ 3 = Good zoonotic TB preventive practice).

4.3.6 Inclusion criteria

- All participants had to be 18 years old and above at the start of the study
- All abattoir workers had to have worked in the abattoir for a minimum of 1 year at the commencement of the study
- Only Fulani pastoralists (herdsmen) at the Kara cattle market in Jos, Plateau State
- Only participants that were able to express themselves took part

4.3.7 Study variables

Independent variables and their categories used in this study were occupation type (Fulani pastoralists, abattoir workers), age group (18-29 years old, 30-40 years old, >40 years old), gender (female, male), level of education (none, primary education, post-primary education) and duration of working experience with livestock (1-3 years, >3 years).

Outcome (dependent) variables used were whether the participants were knowledgeable about bTB or not knowledgeable about bTB and whether they demonstrated good zoonotic TB preventive practice or poor zoonotic TB preventive practice.

4.3.8 Data analysis

4.3.8.1 Descriptive statistics

Data obtained were entered into Microsoft Excel spreadsheets and analysed using Stata/MP version16.0 statistical software. Descriptive statistics was used for the demographic characteristics, knowledge on bTB, and the zoonotic TB preventive practices demonstrated by abattoir workers and Fulani pastoralists. Summary of demographic characteristics was presented in frequency and percentages. Summary on response to questions on bTB knowledge and zoonotic TB preventive practices of participants were presented in frequency, percentages, and their confidence intervals (CIs).

4.3.8.2 Chi squared test

SPSS IBM version 24 software was used to conduct this test. The association between the independent variables and outcome variable was established using the Chi-square test of association. Two outcome variables of whether participants were knowledgeable about bTB or not, and whether participants demonstrated good zoonotic TB preventative practice or not were compared with participant's type of occupation, age group, gender, level of education and duration of working in the livestock sector. $P<0.05$ was considered significant for this test.

4.3.8.3 Univariable logistic regression analysis

Univariable logistic regression analysis was conducted for two outcomes: 1. whether participants were knowledgeable about bTB or not, and 2. whether participants demonstrated good or poor zoonotic TB preventative practice. Input variables considered as part of the

univariable logistic regression models were type of occupation, age group, gender, level of education and duration of working with livestock. Results in the model were expressed as odds ratios (ORs), 95% confidence intervals (CIs) and p-value. Only variables that were significant at P = <0.05 in the univariable model were considered for inclusion in the multivariable model. Data analysis was conducted using Stata/MP version16.0 statistical software.

4.3.8.4 Multivariable logistic regression analysis

In multivariable analysis, in addition to the inclusion criteria, a forward adding and backward removing selection approach was used to select the best fit model based on Akaike Information Criterion (AIC). Results in the model were expressed as odds ratios (ORs), 95% confidence intervals (CIs) and associated p-value. P = <0.05 was considered significant. Data analysis was conducted using Stata/MP version16.0 statistical software.

4.3.8.5 Model checking

The Hosmer–Lemeshow statistic was used to test the goodness of fit of the model. P- value <0.05 was considered significant for this study. Variables rejected at either the univariable or multivariable model-building stages were tested for confounding. Variables were considered to be confounders if their inclusion changed the significance of any other variable, or if the odds ratio of another variable changed by more than 20% (Dohoo et al., 1997).

4.3.9 Ethical considerations

Permission was obtained from the Plateau State Ministry of Agriculture as well as from the management of Jos abattoir and Kara livestock market. The purpose and benefits of the study were explained to participants and verbal consent was obtained from all eligible abattoir workers and Fulani pastoralists. Confidentiality of information was assured.

4.4 Results

4.4.1 Socio-demographic characteristics of respondents

A total of 405 livestock workers participated in this study which included Fulani pastoralist (n = 207, 51.11%) and abattoir workers (n = 198, 48.89%). The median age of respondents was 37 years (Range = 62, IQR = 20). The highest proportion of the participants was within the age group above 40 years (n = 172, 42.47%), males (n = 390, 96.30%), had no formal education (n = 273, 67.41%) and have been working in the livestock industry for more than 3 years (n = 334, 82.47%) (Table 4.1).

Table 4. 1 Demographic characteristics of participants (N=405)

		Frequency	Percentage (%)
Median age = 37yrs (Range = 62, IQR = 20)			
Occupation type			
	Fulani pastoralist (herdsmen)	207	51.11
	Abattoir worker	196	48.89
Age group (years)			
	18-29	127	31.36
	30-40	106	26.17
	>40	172	42.47
Gender			
	Female	15	3.7
	Male	390	96.3
Level of education			
	None	273	67.41
	Primary education	37	9.14
	Post-primary education	95	23.46
Duration of working experience with livestock(years)			
	1-3 yrs	71	17.53
	>3 yrs	334	82.47

4.4.2 Bovine TB knowledge and associated determinants

More than half of the respondents (n = 297, 73.33%, Cl = 68.80-77.43) were aware of bTB ("tarfin fuka"), while few of them (n = 108, 26.67%, Cl = 22.57-31.20) said they were hearing about it for the first time during this study. Among those that have heard of bTB, only few respondents (n = 76, 25.56%, CI = 20.93-30.88) knew that TB is transmissible from cattle to human and far fewer of them (n = 18, 6.06%, Cl = 3.84-9.43) knew of TB transmission from human to cattle. Above a quarter of the respondents (n = 87, 29.29%, CI = 24.38-34.74) knew at least two modes of TB transmission and more than three quarter of the respondents (n = 232,78.11%, 73.03-82.47) could mention at least two signs of TB in cattle (Table 4.2).

Table 4.2 Bovine TB knowledge of livestock workers (Fulani pastoralists and abattoir workers) in Plateau State

	No. of respondents	Response	Frequency (%)	95% Conf. Interval	
I am aware of bTB	405	No	108 (26.67)	22.57	31.2
		Yes	297 (73.33)	68.8	77.43
TB can be transmitted from cattle to human	297	No	221 (74.41)	69.12	79.07
		Yes	76 (25.59)	20.93	30.88
TB can be transmitted from human to cattle	297	No	279 (93.94)	90.57	96.16
		Yes	18 (6.06)	3.84	9.43
Can you mention any two modes of zoonotic TB transmission	297	No	210 (70.71)	65.26	75.62
		Yes	87 (29.29)	24.38	34.74
Can you mention any two signs of TB in cattle?	297	No	65 (21.89)	17.53	26.97
		Yes	232 (78.11)	73.03	82.47

4.4.2.1 Chi squared test of association of bTB knowledge of livestock workers and associated determinants

less than half of the participants (n = 120, 29.63%) were knowledgeable on bTB while more than half of them (n = 285, 70.37%) were not knowledgeable. This was significantly associated with the age group ($\chi 2$ = 54.47, p=<0.001), level of education ($\chi 2$ = 29.58, p=<0.001) and years of working with livestock ($\chi 2$ = 21.07, p=<0.001) of participant, of which many of them that were knowledgeable on bTB were within the age group above 40 years old (n =80, 46.51%), had post-primary education (n = 49, 51.58%) and have worked in the livestock industry for more than 3 years (n = 115, 34.43%) (Table 4.3 and Figure 4.1).

Table 4. 3 Chi squared test of association between bTB knowledge among livestock workers and the type of occupation, age group, gender, level of education and duration of working experience with livestock

		TB Knowledge		Chi square test	
	Category	Not knowledgeable (%)	Knowledgeable (%)	(χ2)	p-value
Type of occupation				1.90	0.168
	Fulani pastoralist (herdsmen)	157 (73.43)	55 (26.57)		
	Abattoir worker	133 (67.17)	65 (32.83)		
Age group (years)				54.47	<0.001
	18-29	118 (92.91)	9 (7.09)		
	30-40	75 (70.75)	31 (29.25)		
	>40	92 (53.49)	80 (46.51)		
Gender				1.98	0.159
	Female	13 (86.67)	2 (13.33)		
	Male	272 (69.74)	118 (30.26)		
Level of education				29.58	<0.001
	None	208 (76.19)	65 (23.81)		
	Primary education	31 (83.78)	6 (16.22)		
	Post-primary education	46 (48.42)	49 (51.58)		
Duration of working experience with livestock				21.07	<0.001
	1-3 years	66 (92.96)	5 (7.04)		
	>3 years	219 (65.57)	115 (34.43)		
Total		285 (70.37)	120 (29.63)		

P<0.05 significant level

4.4.2.2 Univariable logistic regression analysis of factors influencing TB knowledge of livestock workers

This model shows that age group, level of education and duration job in the livestock industry were significantly associated with bTB knowledge. Participants among age groups 30-40 years old (OR = 5.42, CI = 2.44–12.02, P = <0.001) and above 40 years old (OR = 11.40, CI = 5.43–23.92, P = <0.001) were more likely to be knowledgeable on bTB than those below 30 years old. Those that had post-primary education (OR = 3.41, CI = 2.09–5.56, P = <0.001) were more likely to be knowledgeable on bTB than those that had no formal education, and those that have worked in the livestock sector for more than 3 years (OR = 6.93, CI = 2.72–17.61, P = <0.001) were more likely to be knowledgeable on bTB than those with 3 years or less of working experience in the livestock sector (Table 4.4).

Types of occupation (p = 0.169) and gender (p = 0.177) were not found to be significantly associated with bTB knowledge of participants and hence were not included in the multivariable model.

Table 4. 4 Univariable logistic regression analyses of factors influencing knowledge on bTB amongst livestock workers in Plateau State (N = 405)

	Category	Odds Ratio	95% Confidence interval		p-value
Type of occupation					
	Fulani pastoralist (herdsmen)	REF			
	Abattoir worker	1.35	0.88	2.07	0.169
Age group (years)					
	18-29	REF			
	30-40	5.42	2.44	12.02	<0.001
	>40	11.40	5.43	23.92	<0.001
Gender					
	Female	REF			
	Male	2.82	0.63	12.69	0.177
Level of education					
	None	REF			
	Primary education	0.62	0.25	1.55	0.306
	Post-primary education	3.41	2.09	5.56	<0.001
Duration of working experience with livestock					
	1-3 years	REF			
	>3 years	6.93	2.72	17.61	<0.001

4.4.2.3 Multivariable logistic regression analysis of factors influencing TB knowledge of livestock workers

The final multivariable model shows that age group, level of education and years of working with livestock were strong determinants of bTB knowledge. Participants who were within age groups 30-40 (AOR = 4.17, CI = 1.79–9.75, P = 0.001) and above 40 years old (AOR = 8.52, CI = 3.73–19.45, P = <0.001) were more likely to be knowledgeable on bTB than those within age group 18-29 years old. Those that had a post-primary education (AOR = 7.79, CI = 4.05–14.97, P = <0.001) were more knowledgeable on bTB than those with no formal education. Those that have worked with livestock for more than 3 years (AOR = 7.49, CI = 2.49–22.59, P = <0.001) were more likely to be knowledgeable on bTB than those that have worked with livestock for 3 or less years (Table 4.5).

Hence, being within age groups 30-40 years or more than 40 years, having a post-primary education and working with livestock for more than 3 years are strong determinants of bTB knowledge of livestock workers (abattoir worker and Fulani pastoralist) in Plateau State, Nigeria.

No confounding was detected for variables rejected at the univariable or multivariable stages. The Hosmer-Lemeshow goodness of fit test returned a p-value of 0.857, therefore there was no evidence of a lack of fit to the data.

Table 4. 5 Multivariable logistic regression analyses of factors influencing knowledge on bTB amongst livestock workers in Plateau State (N = 405)

	Category	Adjusted Odds Ratio	95% Confidence interval		p-value
Age group (years)					
	18-29				
	30-40	4.17	1.79	9.75	0.001
	>40	8.52	3.73	19.45	<0.001
Level of education					
	None				
	Primary education	1.91	0.67	5.50	0.228
	Post-primary education	7.79	4.05	14.97	<0.001
Duration of working experience with livestock					
	1-3 years				
	>3 years	7.49	2.49	22.59	<0.001

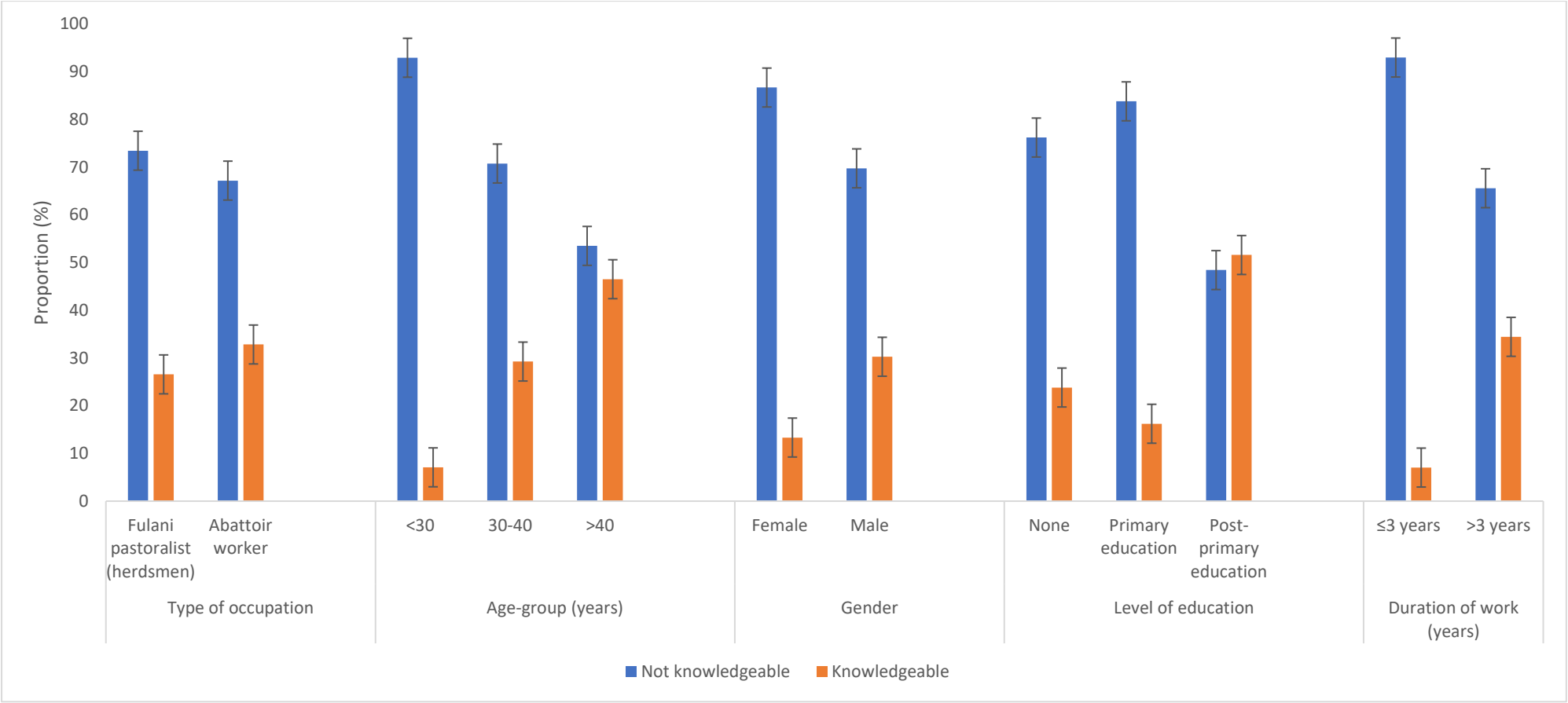

Figure 4. 1 TB knowledge according to occupation type, age group, gender, level of education and duration of work of participants

4.4.3 Zoonotic TB preventive practices and associated determinants

Less than a quarter of the respondents (n =62, 15.31%, CI = 12.11-19.17) admitted to using PPE at work and less than half of them admitted to not cohabiting with cattle (n = 160, 39.51%, CI = 34.84-44.37) and not using the same watering point as cattle (n = 172, 42.47%, CI = 37.73-47.35). Few respondents (n = 27, 6.67%, CI =4.61-9.56) admitted to not consuming meat with lesion nor unpasteurized raw milk nor "nunu" while far fewer of them (n = 14, 3.46%, CI = 2.05-5.76) admitted to being vaccinated with BCG vaccine (Table 4.6). Figure 4.2 show some risky practices that can potentially cause zoonotic TB transmission.

Figure 4. 2 Photographs of some risky practices that may expose humans to zoonotic transmission of tuberculosis

A - selling unpasteurised sour milk ("nunu")

B - Drinking unpasteurised sour milk ("nunu")

C -working in the abattoir without personal protective equipment

Table 4.6 Zoonotic TB preventive practices of livestock workers (Fulani pastoralists and abattoir workers) in Plateau State (N=405)

	Response	Frequency (%)	95% Conf. Interval	
I use PPE to handle livestock and livestock product	No	343 (84.69)	80.83	87.89
	Yes	62 (15.31)	12.11	19.17
I do not cohabit with cattle	No	245 (60.49)	55.63	65.16
	Yes	160 (39.51)	34.84	44.37
I do not use the same watering point as cattle	No	233 (57.53)	52.65	62.27
	Yes	172 (42.47)	37.73	47.35
I do not consume meat with lesion, nor unpasteurized raw milk nor “nunu”	No	378 (93.33)	90.44	95.39
	Yes	27 (6.67)	4.61	9.56
I am vaccinated with BCG vaccine	No	391 (96.54)	94.24	97.95
	Yes	14 (3.46)	2.05	5.76

4.3.3.1 Chi squared test of association of zoonotic TB preventive practices demonstrated by livestock workers and their associated determinants

Regarding practices associated with zoonotic TB transmission, far fewer participants (n = 40, 9.88%) demonstrated good zoonotic TB preventive practice while most of them (n = 365, 90.12%) demonstrated poor zoonotic TB preventive practice. This was found to be associated with the type of occupation ($\chi 2 = 26.48$, P = <0.001), age group ($\chi 2 = 17.16$, P = <0.001) and level of education ($\chi 2 = 48.02$, P = <0.001) of participants of which most of those that demonstrated good zoonotic TB prevention practice were abattoir workers (n =35, 17.68%), within the age groups above 40 (n = 29, 16.86%) and had a post-primary education (n =27, 28.42%) (Table 4.7).

Table 4. 7 Chi squared test of association between zoonotic TB preventive practices among livestock workers and the type of occupation, age group, gender, level of education and duration of working experience with livestock

		Zoonotic TB preventive practice		Chi-square test	
	Category	Poor practice (%)	Good practice (%)	(χ2)	p-value
Type of occupation				26.48	<0.001
	Fulani pastoralist (herdsmen)	202 (97.58)	5 (2.42)		
	Abattoir worker	163 (82.32)	35 (17.68)		
Age group (years)				17.16	<0.001
	18-29	119 (93.70)	8 (6.30)		
	30-40	103 (97.17)	3 (2.83)		
	>40	143 (83.14)	29 (16.86)		
Gender				0.18	0.671
	Female	14 (93.33)	1 (6.67)		
	Male	351 (90.00)	39 (10.00)		
Level of education				48.02	<0.001
	None	262 (95.97)	11 (4.03)		
	Primary education	35 (94.59)	2 (5.41)		
	Post-primary education	68 (71.58)	27 (28.42)		
Duration of working experience with livestock (years)				0.20	0.657
	1-3 years	65 (91.55)	6 (8.45)		
	>3 years	300 (89.82)	34 (10.18)		
Total		365 (90.12)	40 (9.88)		

P<0.05 significant level

4.3.3.2 Univariable logistic regression analysis of factors influencing the zoonotic TB preventive practices demonstrated by livestock workers

This model revealed that the type of occupation, age groups and level of education were significant determinants of participants' zoonotic TB preventive practice. Abattoir workers (OR = 8.67, CI = 3.32-22.64, P = <0.001) were more likely to demonstrate good zoonotic TB preventive practice than Fulani pastoralist. Those that were within the age group above 40 years old (OR = 3.02, CI = 1.33-6.85, P = 0.008) were more likely to demonstrate good practice than those within the less than 30 years old age group. Those that had post-primary education (OR = 9.46, CI = 4.47-20.02, P = <0.001) were more likely to demonstrate good practice than those with no formal education (Table 4.8).

Gender (p = 0.696) and duration of work (p = 0.658) were not found to be significantly associated with zoonotic TB preventive practices and hence were not included in the multivariable model.

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Table 4. 8 Univariable logistic regression analyses of factors influencing zoonotic TB preventive practice amongst livestock workers in Plateau State (N = 405)

	Category	Odds Ratio	95% Confidence interval		p-value
Type of occupation					
	Fulani pastoralist (herdsmen)	REF			
	Abattoir worker	8.67	3.32	22.64	<0.001
Age group (years)					
	18-29	REF			
	30-40	0.43	0.11	1.68	0.226
	>40	3.02	1.33	6.85	0.008
Gender					
	Female	REF			
	Male	1.56	0.20	12.15	0.674
Level of education					
	None	REF			
	Primary education	1.36	0.29	6.40	0.696
	Post-primary education	9.46	4.47	20.02	<0.001
Duration of working experience with livestock (years)					
	1-3 years	REF			
	>3 years	1.23	0.50	3.05	0.658

P<0.05 significant level

4.3.3.3 Multivariable logistic regression analysis of factors influencing the zoonotic TB preventive practices demonstrated by livestock workers

This model also shows that the type of occupation, age groups, and level of education were strong determinants of zoonotic TB preventive practice of participants. Abattoir workers (AOR= 5.13, CI = 1.72-15.27, P = 0.003) were more likely to demonstrate good practice than Fulani pastoralist. Those within the age groups above 40 years old (AOR= 5.22, CI = 1.99-13.68, P = 0.001) were more likely to demonstrate good practice than those within the age groups 18-29 years old. Those with post-primary education (AOR= 9.46, CI = 4.47-20.02, P = <0.001) were more likely to demonstrate good practice than those with no formal education (Table 4.9).

Hence, working in the abattoir, being within age groups more than 40 years and having a post-primary education were strongly associated with good zoonotic TB preventive practice among livestock workers in Plateau state, Nigeria.

No confounding was detected for variables rejected at the univariable or multivariable stages. The Hosmer-Lemeshow goodness of fit test returned a p-value of 0.804, therefore there was no evidence of a lack of fit to the data.

Table 4. 9 Multivariable logistic regression analyses of factors influencing zoonotic TB preventive practice amongst livestock workers in Plateau State (N = 405)

Category	Adjusted Odds Ratio	95% Confidence interval		p-value
Type of occupation				
Fulani pastoralist (herdsmen)	REF			
Abattoir worker	5.13	1.72	15.27	0.003
Age group (years)				
18-29	REF			
30-40	0.43	0.11	1.75	0.239
>40	5.22	1.99	0.24	0.001
Level of education				
None	REF			
Primary education	2.16	0.39	11.84	0.374
Post-primary education	6.29	2.60	15.22	<0.001

P<0.05 significant level

4.4 Discussion

The study describes the knowledge of bTB and zoonotic preventable practices among occupational exposed groups (Fulani pastoralists and abattoir workers) in Plateau State, Nigeria. Such study is vital and in line with the initiation of WHO/OIE/FAO/IUATLD (Road Map for Zoonotic Tuberculosis) to achieve the WHO 2030 End TB strategy.

Most of the livestock respondents were young adults with a median age of 37 years (range = 62, IQR = 20) and most of them were males, had no formal education, and had worked with livestock for more than 3 years. These characteristic findings are similar to the findings of previous studies conducted in Nigeria (Awah Ndukum et al., 2010; Hambolu et al., 2013; Ismail et al., 2015; Kachalla et al.; Sa'idu et al., 2015a).

This study revealed that though most of the livestock workers were aware of bTB, they however did not know of the zoonotic nature of bTB and its modes of transmission. The study revealed that 73% of the participants were aware of bTB known as 'tafin fuka' in the Hausa language which is one of the languages commonly spoken in the study area and just above a quarter (26%) of them knew of TB transmission from cattle to humans. This was relatively low compared to reports in some studies by Awah Ndukum et al. (2010) in Cameroon and Fekadu et al. (2018) in Ethiopia who reported 82% and 95% of respondents were aware of bTB and 81% and 93% of respondents were aware of TB transmission from cattle to humans respectively. The current study revealed that only a small proportion of participants (6%) had knowledge of human to cattle TB transmission which is low compared to the Cameroon study by Awah Ndukum et al. (2010) in which 49.4% of abattoir workers were aware of human to cattle TB transmission. The lack of knowledge of zoonotic transmission of TB was also reported by Onoja et al. (2010) in Nigeria. In that study, 50% of pastoral household members were not aware of zoonotic TB transmission. In the current study, more than a quarter of the respondents (29%) were aware of at least two modes of TB transmission. Again, this was higher

in study by Awah Ndukum et al. (2010) in which 53.8% of abattoir respondents were able to name at least one mode of TB transmission to humans. The current finding of poor knowledge on zoonotic transmission of TB and the modes of zoonotic TB transmission among livestock workers in Plateau State is an important knowledge gap and can pose a challenge to the control of TB in the state.

The risk of zoonotic infections including TB is potentially high among livestock workers due to the close, prolong and direct contact with their cattle during milking, slaughtering, meat processing, assisting delivery and feeding or indirectly through consumption of milk and other animal products.

Many occupationally acquired infections of abattoir workers and pastoralists could be prevented by human practices including consuming unpasteurized milk, eating meat with lesions, cohabiting with cattle, using the same water source as cattle, and not using PPE to handle cattle and cattle product. It was revealed in this study that more than three quarter of the respondents (84.69%) do not use PPE in handling cattle and animal products, and this is similar to findings by Sa'idu et al. (2015a) and (Cadmus et al., 2018). Thus, they are likely to be exposed to the risk of contracting TB and other zoonotic diseases. A study by Adesokan et al. (2013) linked the lack of use of PPE by livestock workers to poor knowledge about the risk of exposure to zoonotic diseases which could be contracted through contact with infected meat and other animal products during animal slaughtering and meat processing. Cook et al. (2017) in Kenya reported that very few slaughterhouses provide PPE for workers and the few workers that use PPE provide them themselves. The cost of PPE can likely be a limiting factor for its use if workers provide them for themselves. In another study undertaken in Ibadan, Nigeria by Ayoola et al. (2017) who interviewed abattoir workers and reported that 96.7% agree that provision of free PPE would serve as an inducement for its use and all the respondents agree that penalties by abattoir associations would enforce using PPE in the abattoir.

As many as 93.33% of the respondents admitted to consuming meat with lesion or unpasteurized raw milk or "nunu". These habits increase the risk for bTB transmission and thus represent an important public health risk. A study by Hambolu et al. (2013) reported 22% of respondents eat *M. bovis* infected parts of the lungs called "*Fuku Elegusi*" in Oko-Oba abattoir in Lagos State, Nigeria. The researcher stated that eating *Fuku Elegusi* is a socio-culturally embedded practice among abattoir workers. In another study in Tanzania conducted by Mangesho et al. (2017), reported that pastoralist slaughter and eat their animals when it is ill or about to die. The researcher stated that this is a common practice with pastoralist as they believe that whatever made the animal ill or caused it to die will not harm them as it was the animal that was targeted and not them. Milk plays an important role in pastoralism; the pastoral group rely on milk and meat for their nutritional intake. Milk is either consumed at home, sold for cash, or exchanged for grain (Barbara, 1988). The transmission of zoonotic TB and other milk borne pathogens is not limited to pastoralists but to the general rural population. Consumption of unpasteurised raw milk and/or "nunu" is a common practice in Nigeria as few Nigerians can afford imported dairy milk which is in the form of packaged powdered or evaporated tin milk. The bulk of milk consumed by Nigerians is supplied by the Fulani pastoralists which has been reported to be highly contaminated with many microbes (Anya and Ozung, 2019). The high degree of preference for consuming unpasteurized raw milk or "nunu" may also be associated with low cost, culture, taste, and ease of availability as pastoralists supply the milk directly to consumers door and it is unlicensed (Amenu et al., 2019; Kristjanson et al., 2010). A study by Addo et al. (2011b) conducted in Ghana and Mangesho et al. (2017) in Tanzania revealed that many respondents were not aware or do not believe that TB and other diseases can be contracted by drinking unpasteurized milk.

The current study revealed that participants use the same watering point as their cattle. *M. bovis* contaminated water, when ingested, may act as a source of potential transfer of the organism

to animals and humans. Some studies have isolated *M. bovis* from water sources used by wild animals and several herds of cattle that migrate together and share watering sources along their way (Duffield and Young, 1985; Oloya et al., 2007; Phillips et al., 2003; Tanner and Michel, 1999). Close proximity to and/or cohabiting with cattle as seen with most of the livestock workers (Fulani pastoralist and abattoir workers) in the current study has become a common practice as cattle rustlings have become a major crime in Nigeria (Olaniyan and Yahaya, 2016). Similar findings were seen in the works of Ibrahim et al. (2012) and Mfinanga et al. (2003a). Safety was the main reason given for cohabiting with cattle in 77.3% of participants in a study by Mfinanga et al. (2003a). These findings are of public health concern as such practices can lead to zoonotic TB transmission. In a study conducted in Ethiopia, *M. bovis* was found in cattle herders that shared shelters with their cattle (Shitaye et al., 2007).

In the current study, most of the respondents said they have not been vaccinated with the BCG vaccine (although there is a possibility that respondents were vaccinated in early childhood and did not know). This revelation is of concern that such a high proportion of these occupationally exposed individuals had not received this vaccination. Myths and misconception regarding vaccination are still rife in most Nigerian communities (Wondifon, 2013). Also, many pastoralists do not know about the vaccine, a pattern seen in a study by Getnet et al. (2019) in Ethiopia in which majority of the pastoralists were not aware of the BCG vaccine. In another study by Bechir et al. (2004), low vaccination coverage was seen among women and children from pastoral communities because they rarely attend health centres for antenatal/postnatal care. Because of the migratory nature of the pastoralist which makes them difficult to reach, they are often excluded from public health care services including immunisations and they are less likely to seek medical treatment due to long distance travel to health facilities (Gele et al., 2009; Schelling et al., 2005; Sheik-Mohamed and Velema, 1999).

The current study found about 30% of livestock worker to be knowledgeable on bTB and about 10% demonstrated good zoonotic TB preventive practice. This is in line with other studies in which bTB knowledge was found to be low among communities that keep or handle cattle (Addo et al., 2011a; Bihon et al., 2021; Kazoora et al., 2016; Kidane et al., 2015; Memon, 2020; Mfinanga et al., 2003a; Munyeme et al., 2010). This can be attributed to absence of local information on zoonosis due to lack of or inadequate communication between human health and veterinary personnel (Cripps, 2000). Mfinanga et al. (2003a) in a study in Tanzania reported that on an average, about 40% of the study participants practised habits that might expose them to TB. Adesokan et al. (2018) in a study involving three states (Ogun, Ebonyi and Sokoto) in Nigeria reported good zoonotic TB preventive practice was demonstrated by 46.9% of livestock workers and in Karachi Pakistan, it was reported that 31.33% of livestock workers demonstrated good zoonotic TB preventive practice (Memon, 2020). Chowdhury et al. (2018), in Bangladesh revealed cattle owners lack knowledge on zoonotic disease preventive practices. The demographic variables of participants that were found to be strong determinant of bTB knowledge were their age group, level of education and duration of work with livestock, while type of occupation, age group and level of education were strong determinants of good zoonotic TB preventive practices. Although there was no difference in the overall knowledge of TB by the different types of occupation (Fulani pastoralist and abattoir workers) in the current study, however abattoir workers were 5 times more likely to demonstrate good zoonotic TB preventive practice then Fulani pastoralist. Similar findings were seen in the works of Memon (2020) in Pakistan and Adesokan et al. (2018) in Nigeria in which abattoir workers were 2.0 times and 2.0 times more likely to demonstrate good zoonotic TB preventive practices than herdsmen respectively. It was shown that the livestock workers gain more knowledge on TB as they advance in age, and this can be linked to increased years of working experience with livestock. Detection of lesion suggestive of TB in slaughtered cattle in the abattoir may raise

awareness on TB as butchers and pastoralist are notified of carcass condemnation. Also, meat inspection officers may inform pastoralist of the clinical signs of bTB and subsequently increasing their knowledge on TB. This suggest that abattoir workers with substantial experience acquire knowledge about the pathologies in tissues that can lead to condemnation of carcasses just by daily observation of activities of the meat inspectors during routine meat inspection. Similarly, those who had a post primary education were more knowledgeable and more likely to demonstrate good zoonotic TB preventive practice than those that had no formal education. These findings are consistent with findings from studies by Ismail and Josephat (2014) in Tanzania and Ismail et al. (2015), Tobin et al. (2013) and Hambolu et al. (2013) in Nigeria. This shows that education contributes greatly to knowledge and practices regarding TB. Unfortunately, most abattoir workers and Fulani pastoralist are often not educated because they spend most of their lives in remote rural area and hence their access to education is limited (Zinsstag and Yosko, 2004). Furthermore pastoralists have not embraced schooling due to their constantly moving and their dependence on juvenile labour (ILO-IPEC, 2013). A feasible TB preventive approach is to raise awareness of zoonotic TB through grassroots health education training programmes to high-risk communities especially in low-income countries where TST and pasteurization of milk are not routinely practiced (Devi et al., 2021; Kazoora et al., 2016; Wedlock et al., 2002).

4.5 Conclusion

The majority of abattoir workers and Fulani pastoralists knew about bTB. However, there were some important knowledge gaps especially in some areas like the zoonotic nature of TB and modes of TB transmission.

Risky predisposing practices, including consumption of unpasteurised milk and “nunu”, consuming meat with lesions, not using PPE in the abattoir, and cohabiting with cattle, were also revealed.

4.6 Limitations of the study

The respond rate of 53.9% of Fulani pastoralist might be considered a limitation to this study because more Fulani pastoralists would most likely increase the study’s representativeness and robustness. However, the study suffers from a nonresponse bias of less than half (46.1%).

The face-to-face interviews which were used in this study might have increased the likelihood of respondents' inclination to give socially acceptable answers. Other methods, such as speaking with and observing the study population may be an alternative.

The convenience sampling used in the study may limit the generalisability of results. However, the findings represent prevailing level of knowledge on bTB and associated risky practices among both abattoir workers and Fulani pastoralists and the results obtained would be useful in designing TB control plans.

Active case finding of zoonotic TB among livestock workers was not carried out, this would have revealed the prevalence of zoonotic TB among livestock workers. Although this was beyond the scope of this study, previous study has reported the presence of zoonotic TB among abattoir workers and pastoralist in Nigeria (Adesokan et al., 2012; Usman, 2016).

4.7 Recommendations

To safeguard the public against the risks of food borne diseases, there is a need to train abattoir workers and advocate for good practice in the abattoirs.

There is a need for a programme for abattoir workers, pastoralists, and the public to raise awareness of zoonotic TB and preventive measures. This could be achieved by creating an

extensive health education programme, including training mobile health workers, that can reach out to the public and especially the pastoralists. This would go a long way towards improving people's understanding of mitigating against practices that may expose them to TB.

Concerted effort is required from all stakeholders in the livestock and public health sectors to implement a TB control program, as the control of zoonotic TB transmission to humans is ultimately linked to the control of bTB in cattle.

CHAPTER FIVE: GENERAL DISCUSSION AND RECOMMENDATION

5.1 Introduction

Bovine tuberculosis is one of the economically important zoonotic diseases globally because of its public health consequences and the high cost of control and eradication. There is limited appreciation of bTB as a problem in Nigeria. This could be a consequence of a failure to implement existing policies on bTB and inadequate bTB control measures in the country. This is largely driven by a lack of evidence and by political and economic reasons such as the high cost of sustaining testing and paying compensation to livestock owners. There is a paucity of information on the prevalence, risk of exposure, transmission, and public health significance of bTB in Nigeria. Furthermore, TB is chronic in nature and does not present as an acute outbreak and because of this, it does not lend itself to easy identification by cattle owners and policy makers in Nigeria as a public health or economic threat.

Animals and human share the same microenvironment. The links between animals and humans as shown by many studies have made the control of the diseases complex (Davis et al., 2017; Friedmann and Son, 2009; Gibbs, 2014; Gray et al., 2007; Kaplan et al., 2009). Zoonotic TB has long been neglected especially in developing countries like Nigeria. This may be due to scarcity of professionals, lack of finances and political will as well as underestimation of the importance of zoonotic TB by the government and private agencies. The economic implications of zoonotic TB, which include the loss of workers' productive time and money lost from condemnation of affected carcasses have been overlooked (Cadmus et al., 2009). The national strategic plan for TB control (NSP-TB) under the Federal Ministry of Health, Nigeria, prioritises health system governance to provide patient-centred prevention, diagnosis and treatment services for TB in humans (FMOH, 2014). The burden of this disease in humans

cannot, however, be fully addressed without considering the animal reservoir and zoonotic transmission at the animal-human interface.

This thesis includes analysis of data of several types: retrospective data on TB from routine post-mortem examination of cattle slaughtered in Jos abattoir (chapter two); retrospective data on patients tested for TB at JUTH, Plateau State (chapter two); detailed examination of 500 selected cattle carcasses and collection of tissues samples suspected to be infected with TB from these selected cattle slaughtered at the Jos abattoir (chapter three); species differentiation of the mycobacteria agent from isolates of suspected TB infected tissues of cattle slaughtered at Jos abattoir using real-time PCR technique (chapter three) and qualitative data derived from interviews of abattoir workers and Fulani pastoralists using a structured questionnaire (chapter four).

5.2 Prevalence of TB in cattle and human in Plateau State Nigeria

Investigation of the prevalence of bTB in Nigeria has not been given much attention despite the high TB prevalence in the human population according to reports by the World Health Organization (Agho et al., 2014). The studies in chapter two and chapter three provide data showing a pattern of occurrence of TB as well as data showing the prevalence of TB in both cattle and humans. We also see the determining factors responsible for influencing TB in cattle and humans in Plateau State (chapter two).

5.2.1 Prevalence of TB in cattle

In a retrospective study in chapter two, a decline in TB prevalence was seen from 2001 to 2016 in slaughtered cattle at the Jos abattoir Plateau State.

The prevalence of TB based on the retrospective abattoir data on routine examination of cattle carcases was 4.0% (chapter two), whereas the prevalence of TB based on detailed examination

of 500 selected cattle carcases was shown to be almost five times higher (19.6%) (chapter three). Reports were also obtained of routine examination of slaughtered cattle conducted by the abattoir meat inspection officer during the study period in chapter three and prevalence of TB in slaughtered cattle was calculated to be 4.02% which is consistent with the TB prevalence report of 4.0% in slaughtered cattle in chapter two. Findings from these studies illustrate a great underestimation of the occurrence of TB in slaughtered cattle in Plateau State. This is similar to a study by Biffa et al. (2010) in which routine abattoir inspections identified 3.5% of carcasses with TB lesions, whereas detailed meat inspection procedures identified 10.2% of carcasses with TB lesions. This study demonstrates that the current abattoir routine inspection protocol is relatively inefficient while the detailed meat inspection protocols for detection of TB lesions in abattoirs are highly sensitive. According to Etter et al. (2006) who undertook a risk assessment on the poor standards of meat inspection in slaughterhouses, the quality of inspection of meat is low, creating high risks of approving TB infected meat as fit for human consumption.

In chapter two (retrospective observational study), the detection of TB in cattle was associated with age group, month, gender, and location. The high TB prevalence seen among older cattle can be related to the increase of exposure to TB that comes with age (Cleaveland et al., 2007; Cook et al., 1996). Another factor may be the reduction in immunity associated with reproduction and being young enough to reproduce. In a study by Kennedy et al. (2002) who reported that the low TB cases in young animals may be associated with the antimycobacterial immunity provided by the predominance of gamma delta (yd) T cells in calves.

5.2.2 Prevalence of TB in humans

In a retrospective study in chapter two, a decline in TB prevalence was seen from 2001 to 2015 in humans. This decline could be due to innovations including economic growth, funding for

health care and a government strategic plan for TB control which includes providing training for personnel on TB control, funding, strengthening of leadership and managerial skills, political commitment from the Nigerian government, presence of TB treatment centres and laboratories across Nigeria, and strengthening of advocacy and communication on TB control and treatment.

In chapter two, TB in humans was associated with age group, month, and location. In humans, TB continues to predominantly affect young adults in their most productive years of life; the 21-40 years-olds bear the heaviest burden in the current study. This working age group maybe exposed to poorly ventilated and overcrowded work conditions which provide ideal conditions for TB bacteria to spread. This is similar to a study by Ukwaja et al. (2011) and Dim and Dim (2013) in Nigeria in which TB was found to be more prevalent among 25-34 year olds. This is in contrast to a study by Frieden et al. (1995) undertaken in New York City which reported TB to be more prevalent among the elderly, which was attributed to the reactivation of latent infection acquired over the years.

5.3 Species identification of mycobacterium in slaughtered cattle in Jos abattoir

The evidence of *M. tuberculosis* infection in cattle highlights a serious public health concern. This suggest that there is transmission of the TB agent from humans to cattle. In many non-industrialized countries, bTB is a public health problem due to persistent direct contact of humans with animal reservoir hosts and consumption of unpasteurized milk and poorly processed animal products. In Nigeria, cattle share both space and resources with humans. This narrows the gap between species aggravating intra- and inter-species interactions, thus enhancing TB transmission through close repeated contacts and food chain.

TB caused by *M. bovis* has been reported as infecting humans (Palmer, 2006; Pavlik et al., 2003; Smith, 1896), while *M. tuberculosis* which is considered to be primarily the cause of TB

infection in humans has also been reported to infect animals especially those in close prolonged contact with humans (Alfonso et al., 2004; Chandrasekharan and Ramakrishnan, 1969; Montali et al., 2001; Oh et al., 2002; Romero et al., 2011; Steele, 1980; Thoen et al., 1981). In chapter three, out of the 500 selected cattle that were inspected using a detailed meat inspection protocol, 98 of them had lesions suggestive of TB. Of the 98 tissue samples each obtained from 98 cattle, 88 of them were found to be infected with MTC. This was confirmed using molecular typing with real-time PCR. Further differentiation of MTC revealed that none of the isolates from the 88 cattle inspected had *M. bovis* nor *M. bovis* BCG. Surprisingly, 79 (89.8%) of the isolates showed *M. tuberculosis.* This high prevalence of *M. tuberculosis* suggests the possibility of human-to-cattle transmission. There is also a possibility of cattle-to-cattle transmission of *M. tuberculosis* in Plateau State. This needs to be further investigated using genotyping to gain a better understanding of the epidemiology of *M. tuberculosis* infection in cattle and their potential to sustain infection in the absence of human reservoir. Animal-to-animal *M. tuberculosis* transmission was reported in various animal species in a case study by Michel et al. (2003) and in experimental studies in cattle by Adam et al. (2010) and by Villarreal-Ramos et al. (2018) who reported that *M. tuberculosis* is attenuated and avirulent in cattle and hence the probability of dissemination is reduced, making infection due to *M. tuberculosis* harder to maintain in cattle.

Since the prevalence of TB in humans is high in Nigeria, there is a risk of animal infection with *M. tuberculosis* and a risk of transmission back to humans. Hence our findings are of public health interest, especially in Nigeria and other countries where laboratory diagnosis of TB is mostly based on smear microscopy which cannot differentiate between *M. bovis* and *M. tuberculosis*.

5.4 Knowledge on bovine TB among abattoir workers and Fulani pastoralists

TB awareness is a very important parameter to be assessed to provide baseline data for policy makers to plan for and deliver an effective TB control and eradication programme. Reports from some studies in Africa have indicated that awareness, attitudes about and perceptions of zoonotic TB determine the increase or decrease in the risk of zoonotic transmission of diseases in communities that have close relationships with livestock and the general public (John et al., 2008; Shirima et al., 2010).

In chapter four, the majority of livestock workers (abattoir workers and Fulani pastoralists) were shown to know about the existence of bTB but there was a lack of knowledge about the zoonotic nature and mode of contracting the disease. The lack of knowledge of the zoonotic nature of TB may be associated with practices which may increase the risk of infection, such as consuming meat with lesion, consuming unpasteurized milk or "nunu", cohabiting with cattle or using the same watering point as cattle and not using PPE to handle livestock and their products. This is similar to some findings in some studies in Africa which also assess the knowledge and practice among high-risk groups. In a rural district in Ethiopia, Amenu et al. (2010) reported a lack of knowledge about the zoonotic transmission of TB and a high prevalence of risky practices was reported. Mfinanga et al. (2003b) reported that 75% of respondents among tribes in Tanzania had poor knowledge of TB and 40% of respondents practice habits that could potentially predispose them to zoonotic TB such as drinking unpasteurized raw milk or eating infected meat and meat products. Another study by Emanuel et al. (2010) reported patchy awareness of zoonoses combined with food consumption habits and poor animal husbandry practices among animal health workers and livestock keepers in Tanzania which are likely to expose them to increased risk of contracting zoonotic tuberculosis. However, some practices like consumption of unpasteurized raw milk or "nunu" or eating TB

infected meat have been reported to be linked mainly to individuals' culture, attitude and behaviour which cannot be changed by either education or training (McIntyre et al., 2013).

Education and raising awareness of practices which reduce risk, such as use of PPE, not consuming unpasteurized milk, not consuming meat with lesions etc., might be highly beneficial in some situations, particularly in the abattoir environment. However, some cultural practices such as consuming raw unpasteurized milk and "nunu" are almost universal in communities in the study area and may be resistant to change through education.

5.5 Conclusions and Recommendations

1. Test and slaughter campaigns that are routinely carried out in industrialized countries may not be feasible in Nigeria due to the transhumance method of cattle production. The control option recommended by the W.H.O. is to sequester infected animals to create disease free zones and then repopulate with disease free animals and also the pasteurization of milk for domestic and commercial use address the problems of reducing the infection rate of bTB in cattle and reducing the impact on humans. However, such measures may not be practical to target bTB in pastoral systems of cattle production.
2. Due to the general absence of documentation of cattle in Nigeria, it is currently not possible to identify the origin of slaughtered animals with lesion suggestive of bTB observed during post-mortem examination in the abattoir. Introduction of measures such as ear tagging and issuing certificates of sale and transport could allow the origin of cattle to be traced. In addition, a database of known herds and their location should be constructed. This may seem difficult especially in moving populations, but transhumance herds are known to usually migrate along well-defined routes (Ben and

Michael, 2002). This system could work for bTB surveillance and in the face of an outbreak, rapid targeted action could then be instigated.

3. Campaigns should be organized to raise awareness of the risk factors of zoonotic TB transmission. The establishment of a well-equipped laboratory for regular molecular characterization of MTC would be of value for TB surveillance in Nigeria. This would allow early detection of disease outbreaks and identification of the species and strains involved and investigations to gain a better understanding of the epidemiology of *M. tuberculosis* infection in cattle and their potential to sustain infection in the absence of human reservoir.
4. Routine testing and regular reassessment of livestock workers awareness on TB are key to the reduction and elimination of the spread of the diseases from humans to cattle and vice versa.
5. The implementation of safety procedures based on Hazard Analysis and Critical. Control Point (HACCP) principles to guard against harmful practices in the abattoir.
6. Consuming raw meat and unpasteurized milk should be discouraged.
7. PPE should be provided free of charge and steps should be taken to make sure its usage is enforced in the abattoir.

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Appendix

Appendix I Pictures taken at Jos abattoir and environment

Appendix II Picture taken of a Fulani pastoralist milking his cow and of cattle at Kara cattle market, Jos




Appendix III Picture taken of a woman selling “nunu” to the public


Appendix IIV Questionnaire

Bovine Tuberculosis in Plateau state

This questionnaire is to access the knowledge on bovine tuberculosis (bTB) and the zoonotic TB preventive practices demonstrated by livestock workers (abattoir workers and Fulani pastoralists) in Plateau State. The data gathered is intended to be used to improve on the current situation with bTB in Plateau State, Nigeria. I hereby solicit your participation in this survey and assure you that all information will be treated in confidence.

Filling the questionnaire at will is considered as your consent.

Section A: Demographic Information

1. Name_____
2. Age _____
3. Gender
   Male
   Female
4. Geopolitical zones (location) in Plateau State
   North
   Central
   South

Section B: Knowledge on bovine TB

5. I am aware of bTB
   No (skip to section c)
   Yes
6. TB can be transmitted from cattle to human
   No
   Yes
7. TB can be transmitted from human to cattle
   No
   Yes
8. Can you mention any two modes of zoonotic TB transmission?
   No
   Yes 1. _____
   2. _____
9. Can you mention any two signs of TB in cattle?
   No
   Yes 1. _____
   2. _____

Section C: Zoonotic TB preventive practices

10. I use PPE
    No

11. I do not cohabit with cattle	Yes No
12. I do not use the same watering point (e.g river, streams, ponds) as cattle	Yes No
13. I do not consume meat with lesion, nor unpasteurized raw milk or “nunu”	Yes No
14. I am vaccinated with BCG vaccine	Yes No

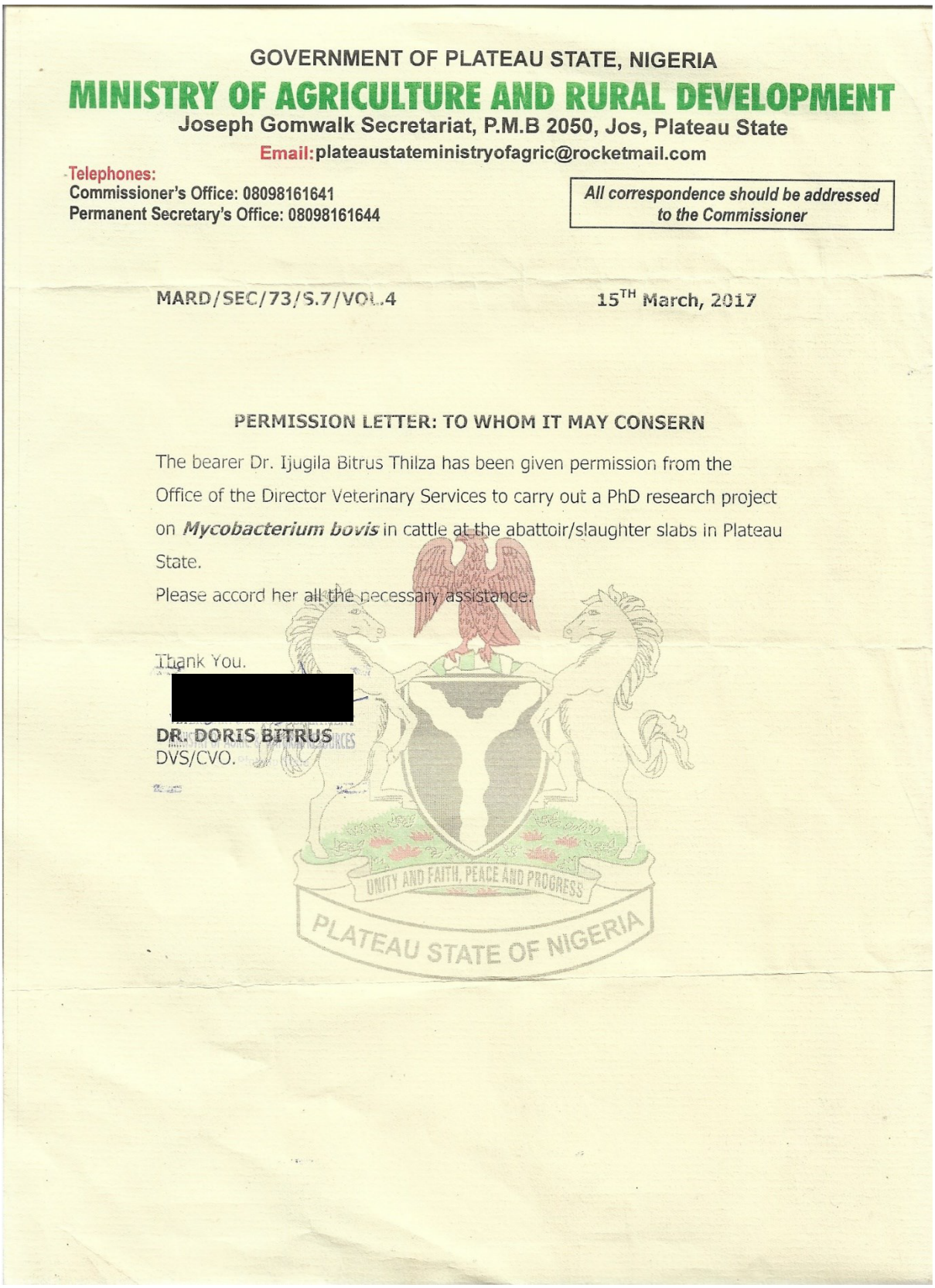
Thank you!

Name of interviewer_____

Signature_____

Date_____

Appendix V Permission letter from Plateau State Ministry of Agriculture and Rural Development to undergo my research in the abattoir.



GOVERNMENT OF PLATEAU STATE, NIGERIA

MINISTRY OF AGRICULTURE AND RURAL DEVELOPMENT

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MARD/SEC/73/S.7/VOL.4 15TH March, 2017

PERMISSION LETTER: TO WHOM IT MAY CONSERN

The bearer Dr. Ijugila Bitrus Thilza has been given permission from the Office of the Director Veterinary Services to carry out a PhD research project on ***Mycobacterium bovis*** in cattle at the abattoir/slaughter slabs in Plateau State.

Please accord her all the necessary assistance.

Thank You.

[redacted]

DR. DORIS BITRUS
DVS/CVO.

UNITY AND FAITH, PEACE AND PROGRESS
PLATEAU STATE OF NIGERIA