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Prevalence and characterisation of *Mycobacterium* species in cattle and sheep at Gauteng abattoirs

By

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List of Abbreviations and Symbols

AEC	Animal Ethics Committe
ARC-OVR	Agricultural Research Council-Onderstepoort Veterinary Research
BCG	*Bacillus* Calmette-Guerin
bp	Base pairbTB Bovine tuberculosis
CFP	Culture filtrate protein
CI	Confidence Interval
CMI	Cell-mediated immunity
CMLE OR	Conditional maximum likelihood estimate of Odds Ratio
CXR	Chest X-ray
DAFF	Department of Agriculture, Forestry and Fisheries
DALRRD	Department of Agriculture, Land Reforms and Rural Development
DLD	Diagnostic laboratory data
DNA	Deoxyribose nucleic acid
dNTP	Deoxynucleotide triphosphates
DOTS	Directly observed treatment short course
DRC	Democratic Republic of Congo
ELISA	Enzyme-linked immunosorbent assay
ESAT-6	Early Secretory antigenic targets
FAO	Food and Agriculture Organisation

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FMD	Food and Mouth Disease
GDARD	Gauteng Department of Agriculture and Rural Development
GKNPC	Greater Kruger National Park Complex
H	HourHCL Hydrochloric acid
HiP	Hluhluwe-iMfolozi Park
HIV	Human Immunodeficiency Virus
HT	High-throughput
HWSETA	Health and Welfare Sector Education and Training
IFN-γ	Interferon-gamma
IgG	Immunoglobulin G
IS	Insertion sequence
KAP	Knowledge, attitude and practices
KL	Kirchner's liquid media
KNP	Kruger National Park
LJ	Loewenstein-Jensen
MAC	*Mycobacterium avium* complex
mg	Milligram
MGIT	Mycobacteria growth indicator tube
ml	Millilitre
MOTT	Mycobacteria other than tuberculosis

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MTBC	*Mycobacterium tuberculosis* complex
n	Number
NA	Not Applicable
NaOH	Sodium Chloride
NCBI	National Centre for Biotechnology Information
NTM	Non-tuberculous mycobacteria
NZG	National Zoological Gardens
OIE	Office International des Epizooties
PCR	Polymerase chain reaction
PPD	Purified protein derivative
PPE	Personnel protective equipment
RMRD-SA	Red Meat Research and Development-South Africa
RD	Region of difference
RNA	Ribonucleic acid
rDNA	Recombinant Deoxyribose nucleic acid
DNA	Deoxyribose nucleic acid
RFLP	Restriction fragment polymorphism
SA	South Africa
SANAS	South African National Accreditation System
TB	Tuberculosis

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TST	Tuberculin skin test
UHT	Ultra-high temperature
UK	United Kingdom
U.S.A.	United States of America
VNTR	Variable number of tandem repeats
WHO	World Health Organisation
ZN	Ziehl-Neelsen
ºC	Degree Celsius
µl	Microliter
%	Percentage

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SUMMARY

Prevalence and characterisation of *Mycobacterium* species in cattle and sheep at Gauteng abattoirs

By

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Bovine tuberculosis (bTB) is a zoonotic disease with a great economic impact estimated at billions of dollars annually worldwide. It is a highly infectious disease infecting mainly wildlife, domestic animals, and humans. The causative agent for the disease is a group of bacteria belonging to the *Mycobacterium tuberculosis* complex (MTBC). The current study aimed at investigating the prevalence and characterize *Mycobacterium* species in slaughter animals at Gauteng province abattoirs and to assess the risk of zoonotic tuberculosis posed to abattoir workers. In an attempt to fulfil the objective samples were collected from Gauteng province abattoirs. There is limited data available on abattoir-based studies on bovine tuberculosis (bTB) in South Africa. Abattoirs were chosen for this study as they provide important information of data and they play a key role in passive surveillance on the status of the prevalence of bTB in livestock.

The study first reviewed the retrospective data for *Mycobacterium* spp. in the laboratory data in the Tuberculosis Laboratory at the Agricultural Research Council-Onderstepoort Veterinary Research (ARC-OVR) between 2007 and 2016. Samples that were submitted at the ARC-OVR from nine provinces of South Africa, were analysed for the prevalence of *Mycobacterium* spp. over these 10 years. The findings revealed that the total prevalence of *Mycobacterium* spp. was 18.47% [834/4516; 95% Confidence Interval: 17.37 – 19.63] with individual species prevalence at 11.47%, 5.20%, 1.53%, 0.24%, and 0.02%, for *Mycobacterium bovis*, Mycobacterium Other Than Tuberculosis (MOTT), *Mycobacterium tuberculosis, Mycobacterium avium,* and *Mycobacterium orygis* respectively. The findings revealed that *M.*

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bovis seems to be the most prevalent *Mycobacterium* species in both domestic animals at 62.26% and wildlife at 63.68%. The study found that factors for bTB such as the presence of wildlife reservoirs and contact with these reservoirs are a great influence on the transmission of the disease that could result in animals testing positive for *Mycobacterium* spp. Samples from Limpopo, Mpumalanga, and Gauteng provinces were most likely positive for *Mycobacterium* spp. The majority of samples from Gauteng province originated from captive wildlife. *M. tuberculosis,* which is mostly a human pathogen rather than *M. bovis,* was isolated. The study also highlights that wild carnivores and marine animals are more likely to test positive as these animals are likely to feed off infected prey. Study results showed that most of the organs were prone to infection by *Mycobacterium* spp. Improved data collection is required so that scientific research can target several aspects highlighted by the information obtained from the records. The laboratory data obtained in this study gave insight into the occurrence of *Mycobacterium* spp. in wildlife, livestock and their environment in South Africa and the factors that influence the transmission of mycobacteriosis such as bTB.
Furthermore, we reviewed serological laboratory data in the Tuberculosis Laboratory at the Agricultural Research Council-Onderstepoort Veterinary Research (ARC-OVR) for the period 2011 - 2016. This was done to further highlight the importance that the combination of both bacteriological data and serology provide better insight into the epidermiology. Out of 10,369 fresh blood samples that were submitted from all of the provinces around South Africa and tested using the Bovigam® kit, the results showed that 1.54% (95% Confidence Interval: 1.30 – 1.78) were positive for bTB, 3.75% (95% Confidence Interval: 3.38 - 4.11) were avian reactors, and 2.29% (95% Confidence Interval: 3.38 - 4.11) were a combination of multiple reactors, equal reactors as well as animals that had a positive screening test. (A multiple reactor means an animal reacted positively to all tuberculin suggesting a possible infection with either *Mycobacterium bovis* or *Mycobacterium forfuitum* or both and in some instances even *Mycobacterium avium*. An equal reactor means an animal reacted positively to both avian or bovine tuberculin due to possible infection with either *Mycobacterium bovis* or *Mycobacterium avium* or both). Also, the results showed that the most number of samples tested were buffalo samples with 88.96% (95% Confidence Interval: 88.35-89.56), followed by domestic bovine (cattle) at 9.94% (95% Confidence Interval: 9.37 – 10.52). Our results showed that there is generally a low prevalence of bTB in South Africa.

Furthermore, we collected fresh blood samples with corresponding tissue samples at the point of slaughter at abattoirs and samples were subjected to serological assays and bacterial culture

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to detect the active interferon-gamma (IFN-γ) and isolate mycobacterial species respectively. The study was conducted at selected abattoirs in the Gauteng Province in South Africa where animals were also subjected to routine meat inspection. A total of 410 fresh blood samples were collected during the slaughter of animals (369 cattle and 41 sheep) from 15 abattoirs and analysed using a Bovigam® 1G-test kit (Prionics AG, Lelystad, The Netherlands) Bovigam test kit with bovine, avian, and *fortuitum* purified protein derivatives (PPD) as blood stimulating antigens. The estimated prevalence of bTB in cattle was 4.4% (95% Confidence Interval: 2.4%-7.3%) and the prevalence of avian reactors was 6.0% (95% Confidence Interval: 3.6%-9.2%). None of the sheep with valid test results (i.e. test samples that have passed quality control checks) were positive for bTB and none were avian reactors (95% Confidence Interval: 0% - 15%).

Additionally, we collected a total of 2000 tissue samples comprising of lungs, liver, spleen, and lymph nodes were collected from 19 abattoirs. Additionally, 19 environmental samples were collected from feedlots, where animals usually drink as they await slaughter. These samples were cultured on Lowenstein-Jensen (LJ) media containing both pyruvate and glycerol. The cultures were monitored for growth over 10 weeks. Colonies that displayed morphology resembling that of *Mycobacteria* were first subjected to Ziehl-Neelsen (ZN) staining, then acid fast bacteria were subjected to a polymerase chain reaction (PCR) assay targeting *Mycobacterium tuberculosis* complex bacteria. No MTBC species were detected by PCR. The same isolates were subjected to the 16S rRNA PCR and gene sequence analysis to investigate and identify non-tuberculous mycobacteria (NTM) species. Isolations were made from eight animals originating from four abattoirs and were identified as *Mycobacterium* species by amplification of a product corresponding to 577 bp in size following gel electrophoresis. Sequence data analysis of the 8 isolates revealed only two of the isolates to be *Mycobacterium colombiense* (99.81% identity) and *Mycobacterium simiae* (99.42% identity). The remaining six isolates were identified as members of the *Actinomadura* species. From the environmental samples, isolation was made from three samples, and two were identified to genus level (*Mycobacterium* species). The remaining isolate was identified as *Mycobacterium senuense* (99.22% identity).

For the slaughtered livestock, the results suggest that there was no risk of transmission of bTB to abattoir workers and the meat was probably safe to consume. Although NTM have been implicated to be potentially involved in causing tuberculosis-like diseases, their rate of occurrence in the current study was extremely low, hence insignificant. The study has,

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however, highlighted that the additional use of serological assays such as the interferon-gamma (IFN-γ) assay can help detect early signs of infection, thus establishing the status of infection.

Additionally, interviews were conducted in the form of questionnaires to establish the knowledge, attitude, and practices (KAP) of abattoir workers regarding Tuberculosis (TB). The objective of the study was to obtain data from abattoir workers in Gauteng Province, South Africa and to assess the risk of zoonotic tuberculosis transmission among workers. This study revealed an overall knowledge score of the respondents to be 42% (95% Confidence Interval: 37.48 - 46.42) ($P<0.001$), with 88.35% of the respondents knowing of the disease. The overall knowledge of TB score was determined by calculating the overall average knowledge of the respondents. More than 45% of the participants were aware of zoonotic TB and how the disease is transmitted. Overall, the results of the study suggested that there are certain practices that abattoir workers are engaged in, such as the consumption of unpasteurized milk and undercooked or raw meat, slaughtering of animals at home as well as taking care of animals at home/work that may promote transmission of zoonotic tuberculosis among themselves and their communities at large.

In conclusion, we report on the prevalence of *Mycobacterium* spp in slaughter animals in abattoirs in Gauteng Province, South Africa, the potential zoonotic risks posed to abattoir workers and on the 10-year retrospective data on samples submitted to the Tuberculosis laboratory (ARC-OVR) analysed for the prevalence of *Mycobacterium* spp. over this 10-year

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1. Chapter 1. Introduction to the study

Bovine tuberculosis (bTB) is considered one of the largest veterinary health problems globally. (Michel *et al.*, 2010). In New Zealand, a study that was conducted in 2004 revealed a prevalence of 0.3% in cattle herds (Ryan *et al.*, 2006). In Ecuador, 4.24% of dairy cattle tested positive for bTB (Proano-Perez *et al.*, 2006). In some African countries, *Mycobacterium bovis (M. bovis)* strains were isolated from Zambia (Munyeme *et al.*, 2009), and Ethiopia (Biffa *et al.*, 2010) and in SA, although the prevalence of bTB decreased to 0.4% in 1995, transmission between livestock was recorded (Michel *et al.*, 2008; Hlokwe *et al.*, 2014; Sichewo *et al.*, 2019). Bovine tuberculosis (TB) is a zoonotic disease, hence it poses a concern to public health (Michel *et al.*, 2010). Organisations such as the Office International des Epizooties (OIE), World Health Organisation (WHO), and Food and Agriculture Organisation (FAO) have classified bTB as a neglected disease, especially in developing countries such as Tunisia (Kahla *et al.*, 2011), and Zambia (Malama *et al.*, 2013). Bovine tuberculosis is a disease with a great economic impact estimated as billions of dollars annually. This is because, for many farmers, cattle are a source of income. The impact is greatly felt in productivity. In SA livestock and wildlife industries are one of the fastest-growing industries and bTB transmissions result in huge economic losses (Michel *et al.*, 2010). From 2000 to 2014 approximately 16,881 cattle with an estimated value of $14 million were culled. Losses are felt not only in livestock production but also in the wildlife industry, as it affects animal trade and international trade (Michel *et al.*, 2019).

World Health Organisations (2021) reported that there was a global drop in the number of people diagnosed with tuberculosis (TB) in comparison with the newly diagnosed people in the year 2019 and that most countries that had a huge drop were India, Indonesia, Phillipines and China. The reason for this was the disruption of TB diagnostics and treatment due to the

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COVID-19 pandemic. As a result of the decrease in the number of people newly diagnosed with TB in 2020 there was an increase in the number of people who died from TB. The regions that had the most TB cases reported in the WHO regions were South-East Asia (43%), Africa (25%), Western Pacific (18%), Eastern Mediterranean (8.3%), Americas (3.0%), and Europe (2.3%). Out of thirty high TB burden countries, eight countries such as India (26%), China (8.5%), Indonesia (8.4%), Philipines (6.0%), Nigeria (4.6%), and South Africa (3.3%) accounted for two-thirds of the worldwide total (World Health Organisation, 2021).

In Africa, the challenge is that the information available on the prevalence of bTB is either not easy to access, fragmented, or incomplete. Additionally, bTB is is not given the required attention in terms of research and diagnosis consequently the true prevalence of the disease is usually because there are numerous gaps in the continents database with information pertaining to prevalence of bTB (Ayele *et al*., 2004; Cousins & Florisson, 2005; Miller, 2015; Dibaba *et al*., 2019). On the other hand, the interference of NTM in the diagnosis of bTB cannot be underestimated (Vordermeier *et al*., 2007; Michel *et al.,* 2011).

Luboya *et al*. (2017) investigated the prevalence of bTB in goats slaughtered at the Kabasele abattoir in the Democratic Republic of Congo (DRC) from the province of Katanga. The prevalence was estimated at 1.7%. While Woldemariam *et al*. (2021) in an abattoir study, established a prevalence of 4.2% for TB in cattle slaughtered the in Adama municipal abattoir in Ethiopia.

In South Africa *M. bovis* was first identified in the Eastern Cape in 1928 in the kudu (*Tragelaphus strepsiceros)* and consequently *M. bovis* was introduced in the Kruger National Park (KNP) into the buffalo population (Paine & Martinaglia, 1929; de Vos *et al*., 1977; Bengis *et al*., 1996). Buffaloes *(Syncerus caffer)* would cross the river to night graze coming into contact with cattle from surrounding farms and this comingling lead to interspecies

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transmission. A follow-up study revealed that there was an epidemiological link between bTB outbreaks in the buffaloes *(Syncerus caffer)* in the KNP and cattle from the neighbouring farms (Michel *et al*., 2008). In Hluhluwe-iMfolozi Park (HiP) the first confirmed case of bTB was in a buffalo (*Syncerus caffer*) followed by infections in bushpigs (*Potamochoerus larvatus*) and baboons (*Papio ursinus*) (Renwick, 2007). In communal cattle sector recently studies revealed that animal prevalences ranging from 0.5%-15% (Musoke *et al*., 2015; Sichewo *et al*., 2015). In South Africa, although several prevalence studies have been conducted in and around the country and within the nine provinces, there is limited data and a lot of gaps concerning the prevalence of bTB in abattoirs. In 1995, the prevalence of the disease nationally was estimated to be below 0.4% and most studies in South Africa have rather focused on specific ecosystems (Michel *et al*., 2008). Bhembe *et al.* (2017) collected 376 lymph nodes samples from slaughtered cattle in two different abattoirs based in the Eastern Cape in SA, *Mycobacterium tuberculosis* complex (MTBC) species were isolated from 162 (43.1%) of the samples.

The current study aimed to determine the prevalence and characterise *M. tuberculosis/M. bovis* and other *Mycobacterium* spp. in slaughter livestock species in abattoirs in Gauteng province and to assess the potential health risk posed to abattoir workers. Abattoirs were chosen for this study because they play a key role in serving as a warning system for future outbreaks and passive surveillance on the prevalence of bTB in livestock. Abattoir-based studies conducted by different authors in several countries reveal an estimate of the status of the prevalence of this disease in livestock, game, and abattoir workers in slaughterhouses (Müller *et al*., 2008; Awah-Ndukum *et al*., 2012; Luboya *et al*., 2017; Mohammed *et al*., 2019).

This study will provide useful information on the current status of the *Mycobacterium* spp. in livestock, and the potential risk of transmission to abattoir workers in slaughterhouses in Gauteng province, SA.

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Problem statement

Tuberculosis is caused by a group of acid-fast, gram-positive bacteria belonging to the *Mycobacterium tuberculosis* complex (Alexander *et al*., 2010). *Mycobacteria* has a wide species range but do not display same virulence in infected host and are divided into maintenance hosts and spillover hosts. *Mycobacterium tuberculosis* is mostly the causative agent in humans while *M. bovis* is the predominant causative agent of tuberculosis in animals. In animals, cattle and buffaloes are the reservoirs of the disease in South Africa (Michel *et al.* 2003; Hlokwe *et al*., 2014; Musoke *et al*., 2015). Tuberculosis is a zoonotic disease with great economic impact estimated as billions of dollars annually especially for farmers as cattle are a source of income. The impact is greatly felt in productivity. The zoonotic potential of the disease is a very important concern to public health (Michel *et al*., 2010). In addition, the interference of non-tuberculous mycobacteria in the diagnosis of bTB cannot be underestimated (Gcebe *et al*., 2017). Due to limited abattoir based studies on *Mycobacterium* in Gauteng Province of South Africa a cross sectional study was conducted in order to determine the prevalence of *Mycobacterium* species in Gauteng abattoirs.

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Chapter 2. Literature review

2.1 History of bovine tuberculosis in South Africa

Bokonyi (1977) suggested that one of the earliest transmissions between animals and man occurred between 7700 and 8000 BC with a domesticated herd of cattle in the basin of the Mediterranean (Bokoyi, 1977). Sheep and cattle that were the source of food mainly facilitated the spread of the disease among humans. Sheratt (1981) proposed that the disease was mainly spread by milk and meat from the sheep that were infected with the pathogen. The first report of bTB in SAwas reported in cattle in 1880 (Hutcheon, 1880). In 1928, bTB was first reported in kudu (*Tragelaphus strepsiceros*) in the Eastern Cape province of this country (Paine & Martinaglia, 1929). In the KNP, bTB was identified in an impala (*Aepycerosmelampus*) (de Vos *et al*., 1977), and in 1990, the bTB causing pathogen was isolated for the first time in the African buffalo (*Syncerus caffer*) (Bengis *et al*., 1996).

2.2 Taxonomic classification

Mycobacteria belong to the order *Actinomycetales* together with other genera such as *Norcadia*, *Rhodococcus,* and *Corynebacterium*. The genus *Mycobacterium* of the family *Mycobacteriaceae* comprises acid-fast, Gram-positive, non-motile, and non-sporulating rods of various lengths, ranging from 0.3 µm to 0.6 µm in diameter and 1.5 µm to 3 µm in length (Koch, 1882; Quinn, 1994). Mycobacteria have a waxy cell wall that makes it difficult for the host's defence mechanisms to penetrate (Taylor *et al*., 2007b). Also, the cell walls of these bacteria consist of mycolic acids that cause them to exhibit characteristics such as resistance to drying, antibiotic resistance, and acid-fastness. It is this unique cell wall construct that results in tuberculosis being such a challenging disease to deal with (Daffe & Draper, 1998). Most mycobacteria are slow-growing organisms and it takes the organisms between 3-10 weeks to

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grow on culture media. However, the rapidly growing mycobacteria may take about a week to grow (Thoen *et al.,* 1981).

Mycobaterium tuberculosis is known to cause TB in humans while *M. bovis* is the predominant causative agent of TB in non-human animals. Both *M. tuberculosis* and *M. bovis* are members of the *Mycobacterium tuberculosis* complex (MTBC). Other species of the MTBC are: *Mycobacterium africanum* (*M. africanun*), *Mycobacterium microti* (*M. microti*), *Mycobacterium pinnipedii* (*M. pinnipedii*), *Mycobacterium caprae* (*M. caprae*), *Mycobacterium mungi* (*M. mungi*) (Alexander *et al*., 2010), and *Mycobacterium canettii* (*M. canetii*) (Feizabadi *et al.,* 1996). The MTBC is a group of mycobacteria with high levels of genetic similarity of up to 99.9% amongst its members (Rogal *et al*., 1990), and it is responsible for infecting different host species. Bovine TB affects a diverse range of species where some species are maintenance hosts of the pathogen and others are spill-over hosts. In South Africa, cattle and buffaloes are the reservoirs of the disease in animals and transmission between animal species has been reported (Michel *et al*., 2003; Hlokwe *et al*., 2014; Musoke *et al*., 2015).

2.3 Pathogenesis

There are several routes through which the infection within and between different species occurs. The major determinant of the infection is greatly influenced by factors such as age, behaviour, environment, and climate. These factors play a very vital role in the process of infection (Pollock & Neill, 2002). The main route of infection is via the respiratory or gastrointestinal tract. If the infection has occurred by inhalation, the bacilli lodge in the respiratory tract, the lungs, and the lymphatic system (Neil *et al*., 1992; Cosivi *et al*., 1995). The effects of infection manifest in the form of lesions that can be found in the lymph nodes, liver, spleen, and other organs (Whipple *et al*., 1996). Therefore, the route of infection in a diseased animal is demonstrated by the location of lesions. Studies have shown that part of the

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reasons why cattle continue to be hosts of *M. bovis*, is because the bacteria are mostly localised in the upper respiratory tract (Corner, 1994). This then makes it easy for open lesions to be aerosolised thus exposing the infection to those that are in close proximity to the infected animal (Costello *et al*., 1998). A study by McIlroy and colleagues (1986) revealed that lesions were observed in 70% of lung and lymph nodes of the respiratory system of positive reactors. It is essential to note that after approximately 14 days post-infection, cell-mediated immunity develops from the lymph nodes (Cassidy *et al*., 1998). Experimental models of bovine tuberculosis demonstrated the involvement of T-cell subsets in response to *M. bovis* infection. The models demonstrated the role of CD4 and CD8 memory T-cells in the production and release of the IFN-γ where their main role is for bacterial containment. (Pollock *et al*., 1996; Cassidy *et al*., 2001). However, if the immune system is unable to contain the bacilli, the infection then starts to spread to other organs (Ayele *et al*., 2004).

2.4 Clinical signs

There are several ways in which infection with the pathogen occurs but the development of the disease is rather a slow process and the clinical signs develop in the advanced stages of disease. After infection, it can take from one year even up to a decade for the infected animal to show symptoms (Renwick *et al*., 2007). The progression of infection to disease is accelerated by factors such as environmental stresses, malnutrition, and aging (Thoen *et al*., 2006). Early in the infection, there are no visible symptoms (de la Rua-Domenech *et al*., 2006). The most common signs include enlarged lymph nodes, chronic cough, lethargy, and anorexia. The second most common form of bTB, are lesions which are seen in advanced stages in necropsied animals (Phillips *et al*., 2003). If infection occurs via ingestion, diarrhoea and constipation may be observed (Cassidy *et al*., 1998; Smith, 2003).

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2.5 Geographical distribution of bovine tuberculosis in South Africa

In Africa there is a relationship that exists between humans and their domestic animals, in small rural country towns more especially home is shared between domestic animals and humans posing a risk for zoonotic TB transmission. In Africa large variations have been reported in bTB occurrence among the various regions. In Africa there is a notion that bTB is more prevalent in humid conditions as compared to drier areas. Also higher bTB prevalences have been reported in peri uban areas as where there is intensive dairy production (Dibaba & Kriek, 2019).

Knowledge of the status of bTB together with its distribution and prevalence is of utmost importance and needs to be accompanied by factors such as legislation and policy to make sure disease control is properly and correctly implemented (Jarvis & Valdes-Donoso, 2018). In Africa, bTB remains a poorly controlled disease, although SA is one of the countries in Africa with a control program that has been sustained over a long period (Dibaba & Kriek, 2019). Bovine tuberculosis is a controlled disease in South Africa according to the Animal Disease Act (Act 35 of 1984). This means that the South African veterinary authorities in 1969, implemented National Tuberculosis Scheme which has regulations dealing with bTB such as diagnosis of bTB, quarantine and slaughtering of infected herds.

In one of the studies in South Africa, Hlokwe *et al.* (2014) used the variable number of tandem repeat and spoligotyping analyses for characterising 490 *M. bovis* isolates from different regions and farms. The isolates were from livestock (cattle, n=230, pig, n=1, and wildlife, n=259). The study revealed that the spatial distribution of bTB has increased in this country and that most outbreaks occurred in the Mpumalanga province of South Africa (Hlokwe *et al.*, 2014).

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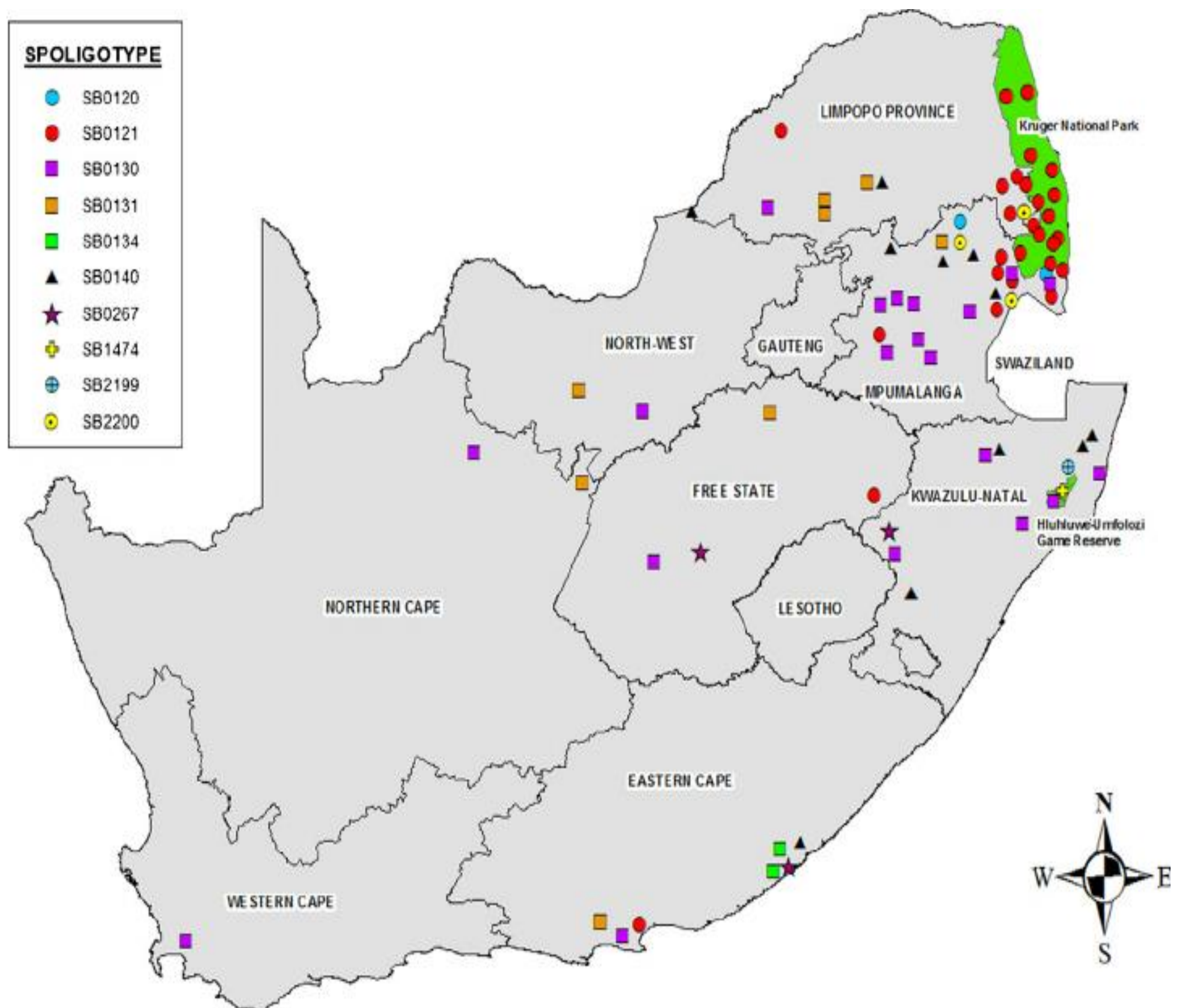
The study also showed that bTB had managed to infiltrate game farms and nature reserves that were previously uninfected in provinces such as Mpumalanga, Limpopo, KwaZulu-Natal, Free State, and North West as shown in Figure 1.1(a) below. During 2000-2014, the National Department of Agriculture, Forestry and Fisheries (DAFF) reported approximately 103 outbreaks of bTB in cattle, as shown in Figure 1.1(b) (Arnot & Michel, 2020).



(a)

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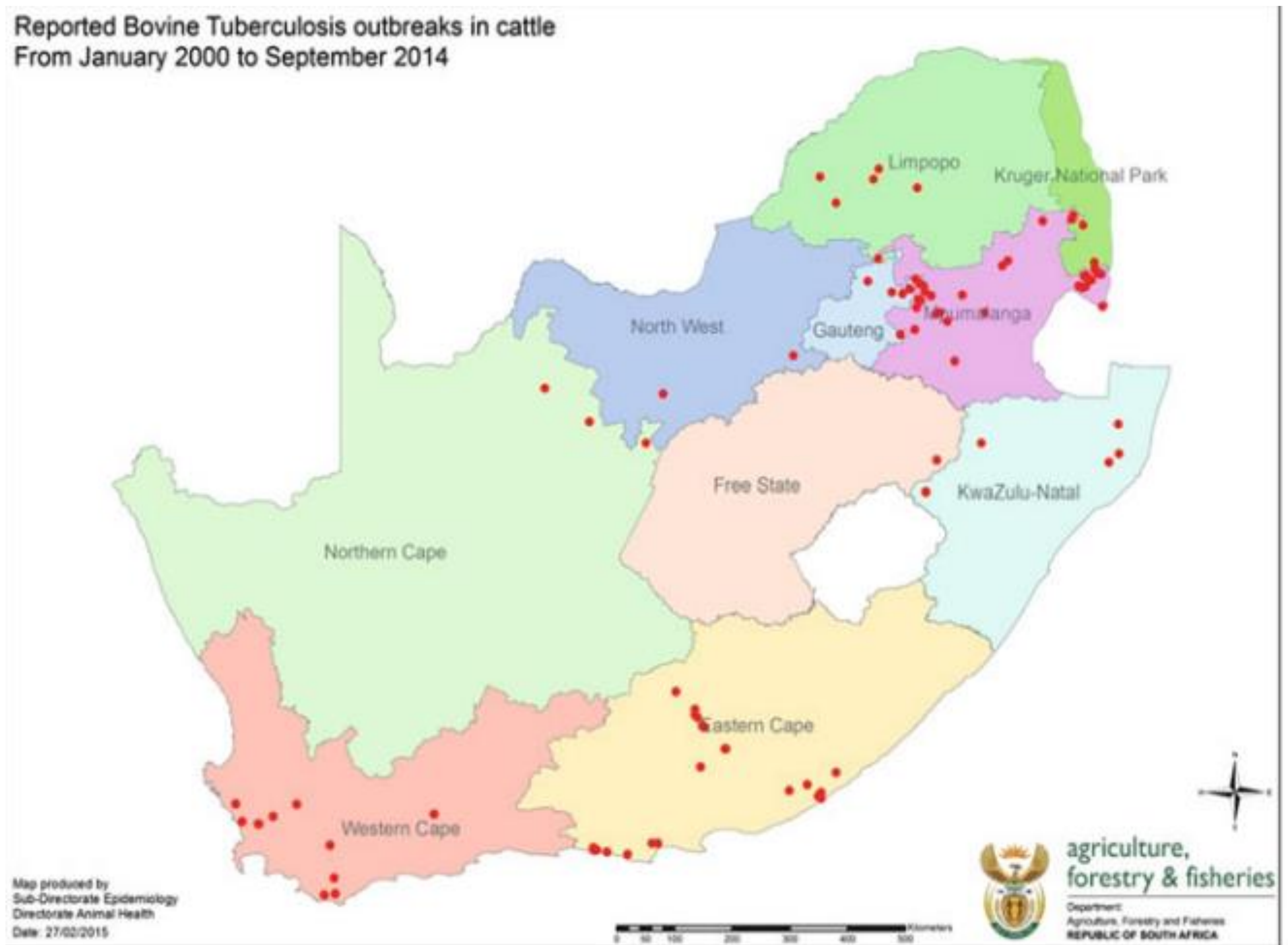

(b)

Figure 1.1: Geographical distribution of (a) *Mycobacterium bovis* strains isolated between 2003 and 2014 in South Africa (Hlokwe *et al.* 2014); (b) Bovine tuberculosis outbreaks in cattle between 2000-2014 (Arnot & Michel, 2020).

The multiple susceptible hosts for bTB make it extremely difficult to manage and control. The other fact that makes it difficult to manage bTB is those wild animals that can harbour mycobacteria for years, which means that animals that have been infected remain in the population for long periods (Renwick *et al.,* 2007; Miller, 2015). This implies that the disease can spill-over to new hosts (Musoke *et al*., 2015). In South Africa, over 21 (Hlokwe *et al*.,

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2014; Michel *et al.*, 2015) wildlife species have been recorded to be infected with *M. bovis* and the first case recorded was in a Kudu (*Tragelaphus strepsiceros*) in the Eastern Cape (Paine & Martinaglia, 1929). Several other species including the duiker (*Sylvicapra, grimmia*), bushbuck (*Tragelaphus scriptus*), and a hare (*Lepus spp*.) were recorded as species that showed evidence of infection by *M. bovis* (Paine & Martinaglia, 1929). The prevalence of bTB in South African game reserves is currently unknown although sporadic infections are reported (Hlokwe *et al*., 2014). In SA, the first confirmed case of an *M. bovis* infection in a game reserve, was in HiPin 1986, where a buffalo was infected (Jolles, 2004). The HiP is recognised as the fourth largest game reserve in South Africa. Spillover of the disease in the same game reserve was documented in species such as the lion (*Panthera leo*) and baboons (*Papio Ursinus*) (Michel *et al*., 2006). In the Madikwe Game Reserve, which is located in the North West Province of South Africa bordering Botswana, *M. bovis* was detected in captured African buffaloes (*Syncerus caffer*) (Hlokwe *et al*., 2016). In the KNP, additional species that were infected with *M. bovis* were recorded, which now included the lions (*Panthera leo*), black rhinoceros (*Diceros bicornis*), cheetahs (*Acinonyx jubatus*), greater kudu (*Tragelaphus strepsiceros*), baboons (*Papio ursinus*), leopards (*Panthera pardus*), and others (Renwick, 2007).

In the early 1990s, the prevalence of bTB in cattle in South Africa was reduced significantly, up until the disease was diagnosed in the African buffalo herds (Renwick, 2007). The definition for free-ranging wildlife species is that they are not enclosed or managed privately. In many countries, it is extremely difficult to completely eradicate bTB with free-ranging maintenance hosts (Miller *et al*., 2013). In the free-ranging swine, *M. bovis* was isolated, and because it is a maintenance host, the probability of spillover infections to other animal species is high. Infections in the free-ranging communities are likely to occur where drinking and feeding areas are shared by the animals (Miller & Olea-Popelka, 2013). A study by Gey van Pittius and co-workers (2012) reported the isolation of *Mycobacterium orygis* (*Oryx bacillus*) from a ranging

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buffalo on a private game farm in KwaZulu-Natal. This was the first time that this microorganism had been isolated in South Africa and some reports suggest that this particular organism can infect humans as well (Duffy *et al*., 2020) . In another study, the microorganism *Mycobacterium suricattae* was isolated from free-living meerkats (*Suricata suricatta)* from the Kalahari Desert (Parsons *et al*., 2013). In a recent study by Hlokwe *et al*. (2019), *M. bovis* was isolated in two giraffes (*Giraffa camelo pardalis*). These giraffes were located in two nature reserves in the Greater Kruger National Park Complex (GKNPC). This report is the first of its kind on *M. bovis* infection in giraffes in South Africa.

2.6 Impact of bovine tuberculosis in Africa

For both wildlife and livestock, losses are incurred in productivity and in the slaughtering and culling of infected livestock and wildlife (Michel *et al*., 2019). Farmers and national parks suffer great losses as most game reserves generate part of their income by selling disease-free animals abroad. If animals become infected, their movement is restricted resulting in losses in revenue for the national parks and farmers (Perry & Grace, 2009). For livestock farmers, approximately 50 million cattle are infected annually worldwide, resulting in economic losses of approximately 3 billion USD (Waters *et al*., 2012). In Morocco, losses as a result of bTB are estimated at $44,260,411, and in Nigeria, out of 61,654 cattle slaughtered from 2008-2012, 1.9% were bTB positive, and in Ethiopia, milk yield was reduced by 5-13% as a result of bTB positive cows (Berrada, 1993; Ameni & Erkihun, 2007; Ejeh *et al*., 2014). In South Africa, during 2000-2014, DAFF reported that 16,881 head of cattle with a value of US$ 14 million were culled. In 2016, in South Africa the wildlife industry and ecotourism contributed 2.9% of the total Gross Domestic Product (GDP) to the South African economy and the mere possibility of infection by *M. bovis* is a threat to the industry and income from tourism. Bovine TB has affected the wildlife industry in SA especially in game parks and farms surrounding the game parks and sporadic outbreaks involving different wildlife species have been reported

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(Hlokwe *et al*., 2014). In a study conducted by Hlowe *et al*. (2016), *M. bovis* infection was detected in African buffaloes (*Syncerus caffer*) destined to be sold. These buffaloes were in the Madikwe game reserve which was previously free of *M. bovis* infection. In an effort to deal with the high prevalence of bTB in dairy herds in 1969 the South African veterinary authorities implemented of the National Bovine Tuberculosis Scheme (Michel *et al*., 2019).

2.7. Transmission of bovine tuberculosis (bTB)

Transmission of *M. bovis* infection occurs in several ways, comprising of animal-to-animal transmission, animal-to-human transmission, and human-to-animal transmission. In animal-to-animal transmissions, cattle are considered the main reservoir for *M. bovis* that causes tuberculosis disease in the animal, hence the name bTB. Infected animals transmit *M. bovis* via secretions such as faeces, milk, saliva, and urine (Neill *et al*., 1991). The disease spreads because of close contact between animals in places such as water points, dipping tanks, and auctions where animals gather, and there is a high possibility of nose-to-nose and mouth-to-mouth contact (Ayele *et al*., 2004). In animal-to-human transmissions, there is a great possibility for *M. bovis*, which is normally known for infecting livestock and wildlife, to infect humans as well just as it is possible for *M. tuberculosis*, which normally infects mainly humans, to infect animals (Michel & Huchzermeyer, 1998). In addition, the practice of keeping wildlife in zoos, game parks, rehabilitation centres, and game reserves, near humans and livestock increases the risk of wildlife-wildlife, human-wildlife, wildlife-livestock, and human-livestock transmissions (Miller, 2013).

Bovine tuberculosis occurs in a wide host range including wild animals, domestic animals, and humans. Species of animals that are infected with *Mycobacterium tuberculosis* complex (MTBC) bacteria are divided into two groups: the maintenance and the spillover hosts or dead-end hosts (Ayele *et al*., 2004). Maintenance hosts can maintain the disease in a population in the absence of a new source of infection, for example, buffaloes, badgers, and deer. In countries

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where there are maintenance hosts, infections are inevitable and not all the species are equally susceptible (de Lisle *et al*., 2002). In Canada, elk (*Cervus Canadensis*) are considered the maintenance host for bTB. Between 1991-2003, outbreaks occurred in 11 cattle herds, which were near a herd of elk (*Cervus Canadensis*). The transmission was suspected to have occurred by indirect contact while feeding on hay bales (Lees *et al*., 2003). In New Zealand, the Australian brushtail possum (*Trichosurus vulpecula*) is the known maintenance host for bTB (Nugent *et al*., 2015) while the white-tailed deer (*Odocoileus virginianus*) is the only reservoir host for the disease in Michigan in the United States of America (O'Brien *et al*., 2011). In SA, the African buffaloes are considered the maintenance host for bTB where they act as wildlife reservoirs (de Vos *et al*., 2001), and in Zambia, the Kafue lechwe antelopes (*Kobus leche Kafuensis*) are the maintenance hosts (Munyeme *et al*., 2009).

Pigs are susceptible to *M. bovis, M. tuberculosis,* and *M. avium*, but most of the infections, in pigs, are caused by *M. avium*. In a study in the Netherlands, *Mycobacterium avium* was isolated from slaughtered pigs originating from the local farms and the average prevalence of lesions was 0.5% (Komijn *et al*., 1999). In a study by Arega and colleagues (2013), the researchers investigated the prevalence of bTB in a region in Ethiopia where such a study had never been done previously. The study was undertaken in slaughter pigs in two abattoirs and the prevalence was found to be around 6%, following *M. tuberculosis* confirmation using RD4 and RD9 typing. Researchers concluded that confirmation of *M. tuberculosis* from pigs is suggestive of transmission between humans and pigs (Arega *et al*., 2013). They also observed that tuberculous lesions were common in older pigs compared to the younger ones and that pigs that were fed in a controlled environment harboured less mycobacterial infections compared to pigs that were left to feed on garbage and were in close contact with humans and other animals. Lesions were mostly observed in the head, liver, lymph nodes, and lungs (Arega *et al*., 2013). In Uganda, a study was carried out to determine the prevalence of bTB in pigs. Out of 997 pigs

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from 31 slaughterhouses, 9.3% were confirmed to be positive for *M. tuberculosis*. This posed a risk, as the infected pigs could be a source of infection to humans (Muwonge *et al*., 2010). In South Africa, *M. bovis* in pigs was detected only twice in the past decade and could have acquired infection from cattle the reason being that pigs and cattle are rarely kept together commercially in the country (Hlokwe *et al.,* 2014).

In a study conducted in Spain on domestic goats, *M. caprae* was identified. *Mycobacterium caprae* seemed to be prevalent in Spanish goats and this was evident in the lesions that it produced (Rodriguez *et al*., 2011). In Nigeria, the presence of mycobacteria in goats was investigated where four strains of *M. bovis,* naturally found in cattle, and one strain of *M. tuberculosis,* were isolated (Cadmus *et al*., 2009). The authors speculated that the possible source of transmission of *M. tuberculosis* was the close cohabitations with humans, thereby resulting in human-goat transmission of the pathogen. In the case of *M. bovis* transmission, the source of infection was speculated to be cattle (Cadmus *et al.,* 2009).

Dogs are susceptible to *M. tuberculosis* and may be infected by *M. bovis* and members of the *M. avium* complex bacteria. Cats, on the other hand, are resistant to *M. tuberculosis* while readily susceptible to *M. bovis.* Poultry can be infected with *M. avium* resulting in avian TB in birds (Dhama *et al*., 2011).

2.8 Tuberculosis in humans

According to the WHO (2017), TB infects one-third of the human population, almost two billion people. In 1993, a global emergency was declared by the WHO because of the scourge of the disease. By the year 2020, TB was projected to be the number one killer on the list. This resulted in 20-30% of household income being lost, putting pressure on the government's health budget (World Health Organisation, 2017). According to the latest WHO (2021), the

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latest report, TB is the 13th leading cause of death globally and ranks above HIV (Human Immunodeficiency Virus) as a top cause of death from a single infectious agent globally.

In humans, TB is mainly caused by *M. tuberculosis*, which is a member of the *M. tuberculosis* complex. The route of infection in human-to-human transmission is the inhalation of air particles contaminated with the bacteria until it reaches the lungs. After the formation of phagosome, the infected macrophages display the *M. tuberculosis* antigen and travel to the mediastinal lymph nodes for the activation of $CD4^+$ T-helper cells. These cells regulate the secretion of cytokines. Thus, tuberculosis in humans is a pulmonary disease spreading from the lungs to the lymphatic system, bloodstream, kidneys, and other distant organs. However, the infection with *M. tuberculosis* normally progresses to TB disease (LoBue *et al*., 2010).

Individuals that are immunocompromised have a greater risk of the infection progressing further into clinical human tuberculosis. Human Immunodeficiency Virus (HIV)-infected persons are more vulnerable to TB because mycobacterial infections are considered opportunistic infections and this becomes a public health threat (LoBue *et al*., 2010). According to the WHO, the high prevalence of HIV has increased the incidence of TB (WHO, 2009). This is evident in countries like South Africa and Swaziland where every year 1% of the population is infected with *M. tuberculosis* because people infected with the HIV are at a risk of being infected with *M. tuberculosis* due to their immunocompromised status. (Lawn *et al*., 2011).

2.9 Zoonotic tuberculosis (TB)

At a zoo in New York Zoological Park, 24 zookeepers were reported to have been exposed to an *M. bovis*-infected white rhinoceros. As a result of the urine, mucus, and faeces on the floor, the cleaning of the contaminated barn resulted in aerosol transmission from animals to humans (Dalovisio *et al*., 1992). Human TB due to *M. bovis* is mostly a result of the consumption of unpasteurised milk, milk products, and exposure to infected aerosol-borne particles. Groups

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that are at risk of being infected are the abattoir workers, farm workers, and veterinarians (Ayele *et. al*., 2004). The most important challenge to defining the extent of transmission of *M. bovis* to humans includes the existing poor surveillance and the fact that that several diagnostic laboratory proceduresare unable to differentiate between *M. bovis* and *M. tuberculosis* using molecular methods (Getahun, 2017). There is, therefore, the assumption that all human cases of TB are due to *M. tuberculosis,* resulting in inaccurate data that may not reflect the true status of zoonotic tuberculosis, which may be under-reported (Olea-Popelka *et. al.,* 2016). Human tuberculosis is enhanced by several factors including HIV, poverty, and limited healthcare facilities (Ayele *et al*., 2004).

In 2006, Ireland reported that out of 400 cases reported for TB, five, were due to *M. bovis.* Whereas, in the UK during the period 1990-2003, human TB due to *M. bovis* was reported to be between 0.5%-1.5% (de la Rua-Domenech, 2006; Bilal *et al*., 2010). Bilal and co-workers reported a case study of a patient that was infected by *M. bovis* from animals on her farm (Bilal *et al*., 2010). Hlavsa *et al.* (2008) collected surveillance data on TB due *to M. bovis* in the United States of America from 2004-2005 and selected data between 1995-2003. The data revealed that 1.4% of human tuberculosis was due to *M. bovis* and the Hispanic population along the borders of Mexico was mostly affected. The report suggested that transmission was due to drinking of unpasteurised milk and consumption of contaminated dairy products. In France, human to human transmission of TB due to *M. bovis* amongst father and daughter was documented by Sunder *et al.* (2009) and both individuals were immunocompetent. The father worked in the slaughterhouse and was occupationally exposed while the daughter could have been possibly infected by the father. The presence of *M. tuberculosis* was confirmed by polymerase chain reaction (PCR). Zoonotic tuberculosis was also reported in Pakistan in abattoir workers and livestock farmers (Khattak *et al*., 2016).

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2.10 Transmission of tuberculosis (TB) from humans to animals

Mycobacterium tuberculosis complex species known to infect humans can also infect livestock and wildlife species. *Mycobacterium tuberculosis*, *M africanum, and M. canetti* infect mainly humans, but spillover infections into other species can occur. A study by Michel & Huchzermeyer (1998) demonstrated a case where *M. tuberculosis* was transmitted from the owner to a pet monkey. Another study by Michel and colleagues (2003) demonstrated the transmission of *M. tuberculosis* from visitors to animals in the NZG of South Africa. In a recent study, Hlokwe and co-workers (2017), reported the isolation of *M. tuberculosis* from cattle originating from two different farms in SA. This was the first report of *M. tuberculosis* infection in cattle from SA (Hlokwe *et al*., 2017). A report by Miller *et al.* (2019) confirmed a case of *Mycobacterium tuberculosis* in a free-ranging African elephant (*Loxodonta Africana*). The report suggests that possible transmission could have occurred through contaminated human waste in settlements near the park or through contaminated food discarded by visitors. *Mycobacterium tuberculosis* has also been reported in rhinoceroses (*Rhinocerotidae*), tapirs (*Tapirus terrestris*) and bovine species (Montali *et al*., 2001).

2.11 Bovine tuberculosis in captive wildlife species in South Africa and other countries

Between 1991 to 2001, *M. tuberculosis* infected eight different species in the National Zoological gardens in Pretoria in South Africa. Infected species included the lesser kudu (*Tragelaphus imberbis*), chimpanzees (*Pan terrestris*), reedbuck (*Redunca fulvorufula*), baboon (*Papio ursinus*). The source of transmission was thought to be visitors to the zoo or even employees at the zoo (Michel *et al*., 2003). Michel and colleagues in 2013 conducted a follow-up study on the cases of tuberculosis in the National Zoological Gardens between 2002-2011. They discovered tuberculous lesions in 12 species including chimpanzee (*Pan troglodytes*), warthogs (*Phacochoerus africanus*), bongo antelope (*Tragelaphus eurycerus*),

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babirus pig (*Babyrousa babyrusa*), Brazilian tapir (*Tapirus terrestris*), Malayan tapir (*Tapirus indicus*), two beavers (*Castor canadensis*), Patas monkeys (*Erythrocebus patas*), nyala (*Tragelaphus angasii*) lesser kudu (*Tragelaphus imberbis*), gorilla (*Gorilla gorilla*) and an eland (*Tragelaphus oryx*). The risk of transmission in captive wildlife is increased by wildlife that are maintenance hosts of the disease, living near humans and livestock (Miller & Olea-Popelka, 2013). *M. tuberculosis* has been reported in India in elephants living close to humans, this is a result of a competition for resources between the elephants and humans. In a study by Zachariah and colleagues (2017), three out of 88 elephants that were tested by PCR and genetic sequencing were infected with *M. tuberculosis*. The presence of *M. tuberculosis* was confirmed by polymerase chain reaction. Close interactions between human and captive elephants is one plays a major role the interspecies transmission of TB. In North America, the detection of *M. tuberculosis* in captive Asian elephants (*Elephas maximus*) has been recorded since the early '90s (Mikota, 2008). Feldman *et al.* (2013) revealed that TB in captive Asian elephants was higher than that of the African elephants. This was attributed to the close association of these animals and humans. Various studies have recorded zoonotic transmission of *M. tuberculosis* in captive elephants (Michalak *et al*., 1998; Zlot *et al*., 2016).

2.12 Non-tuberculous Mycobacteria (NTM)

Non-tuberculous mycobacteria (NTM) are the mycobacteria other than the ones categorised in the *Mycobacterium tuberculosis* complex. There are approximately 150 species of NTMs that have been described (Johnson & Odell, 2013). There are two groups of NTMs, fast and slow growers and they are found in the environment, soil, and water (Djaibe *et al*., 2006). Non-tuberculous mycobacteria have the potential to infect humans and animals mainly through the digestive route (Kankya *et al*., 2011) and the inhalation of contaminated particles (Courtenay *et al*., 2006). *Mycobacterium marinum*, *M. fortuitum,* and *M. chelonae* normally infect fish, while species such as *M. scrofulaceum*, *M. xenopi*, *M. marinum*, *M. kansasii*, *M. simiae,* and

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M. porcinum infect animals. People infected with HIV are more susceptible to infection by NTM's as their immune system is compromised (Bercovier *et al*., 2001) and are thus regarded as opportunistic pathogens (Mirsaeidi *et al*., 2014). Infection by NTMs cause the same symptoms as the classical TB bacteria and they exhibit resistance to antimicrobial drugs (Djaibe *et al*., 2006). In most cases, the NTMs have the potential to cause extra-pulmonary diseases. *Mycoabctaerium avium* complex (MAC) is the major organism responsible for pulmonary diseases followed by *M. kasassii* and *M. abscessus* (Johnson & Odell, 2013). A study by Michel and co-workers (2008) showed that exposure of both the African buffaloes (*Syncerus scaffer*) and cattle to environmental mycobacteria results in the sensitisation towards bovine and avian PPD in an IFN-γ assay (Michel *et al*., 2008). This means that their exposure to environmental mycobacteria causes cross-reactive immune responses, which interferes with the specificity of the IFN-γ test (Michel, 2008; Schiller *et al*., 2010).

Since NTM's are considered opportunistic pathogens, their infections are of particular concern in countries where HIV is endemic (Falkinham, 1996; Biet & Boschiroli, 2014; Miraeidi *et al*., 2014). The diagnosis of the NTM is also very difficult because they occur in the environment as contaminants (Johnson & Odell, 2013).

Non-tuberculous mycobacterial infections are detected and characterised using methods such as polymerase chain reaction (PCR) and sequencing of the 16S rRNA (Harmsen *et al*., 2003; Gcebe *et al*., 2013). A study by Katale *et al.* (2014) was conducted in the Serengeti ecosystem. Out of 472 sputum samples from suspect TB patients and 606 tissues from wildlife and cattle fifty-five NTM isolates representing 16 mycobacterial species and 5 isolates belonging to the MTBC were detected. The study revealed that *Mycobacterium intracellulare* which was isolated from humans, cattle, and wildlife, was the most frequently isolated species (20 isolates, 36.4%) followed by *M. lentiflavum* (11 isolates, 20%), *M. fortuitum* (4 isolates, 7.3%) and *M.*

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chelonae-abscessus group (3 isolates, 5.5%). Cattle with a previous infection with *M. bovis*, had tuberculous lesions caused by NTM (Katale *et al*., 2014).

In another study, by Gcebe and Hlokwe (2017), NTM species circulating in South African wildlife ecosystems were characterised using PCR and sequencing of the 16S rDNA segment. Of the 102 isolates originating from South Africa and Botswana, 30 species of NTM's were detected (Gcebe & Hlokwe, 2017). Non-tuberculous mycobacteria such as *M. absessus subspecies bolletii, M. terrae* are capable of co-infection with *M. bovis*, indicating that these NTMs have the potential to become opportunistic pathogens (Gcebe & Hlokwe, 2017).

2.13 Diagnosis of tuberculosis in animals

The identification of the mycobacterial species is very important as it facilitates disease management and control. Diagnosis plays a very important role in the surveillance of TB (Michel *et al*., 2010). There are a variety of tests available for the diagnosis of TB in both humans and animals such as cell-mediated immune and humoral mediated based tests, culture and PCR based tests.

2.13.1. Cell-mediated immune-based tests

The main aim of the cell-mediated immune tests is to detect early-mediated immune response to tuberculosis infections (Cousins & Florisson, 2005). The animal and human systems use two types of immunity to identify and destroy foreign antigens; i.e. the cell-mediated and humoral immunity (de la Rua-Domenech *et al*., 2006; Michel *et al*., 2010). Cell-mediated immunity uses T-cells and humoral immunity uses B-cells for the detection of mycobacterium infections (Cooper, 2009). The cell-mediated immune tests are divided into two: the tuberculin skin test (TST) and the IFN-γ test (Bernitz *et al*., 2021).

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2.13.1.1. Tuberculin Skin Test in animals

The TST is a screening tool for the detection of tuberculosis in the early stages of infection (Michel *et al*., 2010; Kriek *et al*., 2019). The principle of the TST is the same for both animals and humans. In animals, there are two types of tuberculin normally used, the avian and bovine tuberculin (Department of Agriculture Forestry and Fisheries, 2017). Tuberculin is composed of protein material that has been isolated from the mycobacterial cell wall. After the injection of tuberculin into an infected animal, it takes 48-72 hours for the inflammatory response to occur, in the case of an animal that has been exposed to the *Mycobacterium* of interest (Snider, 1982; Monaghan *et. al*.,1994). There are two types of skin tests, the single intradermal test, and the comparative test (Keet *et al*., 2010; Kriek *et al*., 2019). With the single intradermal TST, only the bovine tuberculin is used whereas with the comparative TST both the avian and the bovine tuberculin are used and injected injected on the side of the neck, midway between the head and the shoulder and halfway between the top and bottom of the neck. In South Africa this is the only approved site for intradermal TB testing in cattle (DAFF, 2013). In practice, an injection site is located, injected with tuberculin, and if the swelling at the bovine tuberculin injection site exceeded the increase in swelling at the avian tuberculin injection site by 4 mm (Comparative TST), 72 h post-injection, then the animals are considered as positive reactors (Ameni *et al*., 2008). In single TST, animals with skinfold thickness of ≥6 mm at the site of injection are classified as positive reactors (Vekemans *et al*., 1999; Kriek *et al*., 2019). The main disadvantage of TST skin tests is that they cannot differentiate between several species of the complex nor differentiate between vaccinated and non-vaccinated animals (de la Rua-Domenech *et al*., 2006).

2.13.2. Interferon-gamma test

The IFN-γ test is used for measuring the amount of IFN-γ released by the T-cells (Michel *et al*., 2010). Therefore, animals that are not infected will not release the IFN-γ. However, because

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of the sensitisation of animals to certain NTM's, there may be cross-reactions, and false positives when tested (Michel, 2008). This is due to animals that have prior exposure to environmental mycobacteria (Pollock & Anderson, 1997). Several studies revealed that exposure to environmental mycobacteria may result in cattle and buffaloes being sensitised to bovine and avian PPD resulting in reactions in immune response and reducing the specificity of the IFN-γ test (Biet *et al.*, 2014). A study by Michel (2008) also showed that prior exposure to *M. fortuitum* also induced a cross-reactive immune response to *M. bovis* antigens resulting in false-positive results. A recommendation was therefore made from this study that Fortuitum PPD (a PPD prepared from *Mycobacterium fortuitum* strain ATCC 6841) be added to the IFN-γ assay to improve the specificity of the assay (Michel, 2008).

Furthermore, Pollock and Andersen (1997) suggested the use of a single purified antigen such as 6Kda early secretory antigenic targets (ESAT-6) and 10 kDa culture filtrate protein (CFP-10) for improved diagnosis. This study showed that the advantage of using ESAT-6 is for increased specificity as this gene was initially thought to be absent in most environmental mycobacteria and BCG strains but present in the pathogenic species of the MTBC complex (Pollock & Anderson, 1997). However, it has since been found that genes encoding for ESAT-6 and CFP-10 proteins are found in other non-pathogenic as well as pathogenic NTMs such as *M. smegmatis, M. nonchromogenicum*; *M. kansasii,* and *M. marinum* (Vordemeir *et al*., 2000; van Pittius *et al.,* 2001; Gcebe *et al*., 2016).

With PPD based assays, blood samples collected are stimulated with antigens within 8 h of collection. In response to these antigens, IFN-γ is released in the plasma supernatant, and the level is measured by the enzyme-linked immunosorbent assay (ELISA), using the Bovigam kit (in cattle, sheep, goats, and buffalo) (Rothel *et al*., 1990; Wood *et al*., 2001).

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2.14. Humoral mediated based tests

During the late stages of the disease, the early cell-mediated immune response gradually decreases, and the humoral immune response increases (Pollock *et al*., 2002). At this stage, the Bovigam test and the TST are not effective at identifying infected animals, but serological tests which detect the presence of antibodies to the disease of interest are used (Carneiro *et al*. 2021).

2.14.1. Serological tests

Serological tests have been developed for the detection of antibodies against TB (Lekko *et al*., 2020). As a result of recently available antibody technology, there has been a renewed interest in the use of this method (Jolley *et al*., 2007; Ross *et al*., 2016). With the new technology such as new generation sequencing (Dippenaar *et al*., 2017; Roos *et al*., 2018;) that allows for the sequencing of the whole genome of the TB pathogen, the availability of expressed proteins has revolutionised TB testing, thus exploiting the antigen-antibody technologies. (de Lisle *et al*., 2002).

A study by Marassi *et al.* (2011), used two capture antigens MPB 70 and MPB 83 in two indirect IgG ELISAs for the diagnosis of bTB on a dairy herd of cattle. The indirect ELISA proved to be a good complementary assay to identify infected cattle (Marassi *et al*., 2011).

2.15. Direct method of examination

2.15.1. Ziehl Neelsen Staining

This test is based on the staining and microscopic examination of the smears directly from sputum samples or infected organs of carcasses such as lungs and lymph nodes that show tuberculous lesions, or from the bacteria isolated from these specimens (Miller *et al.,* 2016). Smears are stained using Ziehl-Neelsen (ZN) staining which is used for the identification of acid-fast mycobacteria (Bernitz *et al*., 2021). Acid-fast mycobacteria retain the primary stain,

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which is carbolfuchsin, and are not decolourised by the acid while non-acid-fast cells will be decolourised and take up the counter stain; methylene blue (Lekko *et al.,* 2020). A positive ZN stained bacteria consist of red rods due to the retention of the primary stain thus confirming the presence of mycobacteria (Burke & Barnes, 1929). It has been reported that auramine O staining may be more sensitive than the Ziehl-Neelsen staining method (Michel *et al*., 2010). Auromine O is a fluorescent stain that is used for staining the acid-fast bacilli. Mycobacterial cell walls are composed of the mycolic acids. The dye then binds to the mycolic acid giving them a yellow to orange colour when viewed under the microscope (Mote *et al*., 1975; Smithwick *et al*., 1995).

2.15.2. Histopathology

Histopathology is the examination of suspected tuberculous lesions (which maybe may be localised in various tissue orgarns such as liver, lung spleen and lymph nodes) to provide correct diagnosis of TB (Lekko *et al*., 2020). Various histological sections are prepared for the detection of suspected tuberculous lesions (Silva *et al*., 2018). The histological pictures of lesions differ with different hosts. A histological picture of a TB lesion in hosts such as cattle will have central necrosis with mineralisation while TB lesions in carnivores such as lions and leopards, lack giant cells and mineralisation (Varello *et al*., 2008; Kriek *et al*., 2019). The downside of histopathology of TB lesions is that lesions due to *M. bovis* may be mistaken for lesions caused by NTM's (Thoen *et al*., 2009).

2.15.3. Culture of mycobacteria

Culture is still considered as the gold standard for TB diagnosis and is usually a prerequisite for isolating the microorganism (Liebana *et al*., 2008; Kedir *et al*., 2018). Loewenstein-Jensen (LJ) solid media is used for the cultivation of mycobacteria (Gormley *et al*., 2014). After the inoculation of the LJ media, culture slopes are incubated at 37 °C for up to 10 weeks (tissue samples), samples from pigs and birds are incubated at 45°C for 10 weeks and environmental

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samples at 27 °C for 10 weeks (Gormley *et al*., 2014). Any growth on the culture media is further tested by Ziehl-Neelsen staining for acid-fastness (Valerro *et al*., 2008). Acid fastness suggests the presence of mycobacteria. The disadvantages of this test are that it is time consuming and does not differentiate between the different mycobacterial species (De la Rua-Domenech *et al*., 1996; Michel *et al*., 2010).

While solid media are used for observing the morphology of the cultures, liquid media are also used for increased sensitivity and can support large culture inoculum (Michel *et al*., 2010). The Mycobacteria growth indicator tube (MGIT), Kirchner's liquid media (KL), MB Redox tube, and the VersaTrek automated liquid culture systems are examples of the liquid culture media that are used for the detection of mycobacterial growth (Chihota *et al*., 2010).

2.16. Molecular-based tests

2.16.1. Polymerase chain reaction (PCR)

The PCR assay is a sensitive but expensive method and is used for differentiating among the species of the *Mycobacterium tuberculosis* complex species (Parsons *et al*., 2002). Upon confirmation of acid-fastness, the cultures are usually subjected to PCR assays to differentiate between the mycobacteria other than tuberculosis (MOTT) and *M. tuberculosis* complex (MTBC) species (Parsons *et al*., 2002; Hlokwe *et al*., 2014). Isolates that belong to the MTBC are further differentiated by PCR, using primers that target regions of differences such as the RD4, RD9and RD12 (Warren *et al*., 2006). Non-tuberculous mycobacteria are characterised and differentiated by PCR amplification and sequencing of the 16S rRNA gene (Harmsen *et al*., 2003; Gcebe *et al*., 2013). A recent study by Hlokwe and Mogano (2020) used the latest technology, the Xpert® MTB RIF ultra assay, for the detection of MTBC species. This assay is a semi-quantitative, nested real-time PCR assay, and displayed diagnostic sensitivity of 95.24% and specificity of 82% when a comparative evaluation with a culture test was done (Hlokwe & Ratanna, 2020). Furthermore, it was able to detect 18.5% of samples that were

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culture negative. Overall, the results were available in two hours as compared to the 8-10 weeks turn-around time for culture results (Hlokwe & Mogano, 2020).

2.16.2. DNA fingerprinting

Typing methods such as spoligotyping and restriction fragment polymorphism (RFLP IS*6110*) based on the polymorphic and cytosine sequences, a region of repeat sequences and IS*6110* have been used for differentiation between strains of MTBC (de Lisle *et al*., 2002). The IS*6110* is an insertion known to be found only within the members of MTBC (van Embden *et al*., 1993). This insertion sequence is found at different locations and in different copy numbers within the genome of different isolates (Skuce & Neill, 2001). This characteristic has been used mainly in diagnostics for the genotyping of strains (Thierry *et al*., 1990; Coros *et al*., 2008). Therefore, the differences in the genetic variations concerning the location and number of copies of the insertion sequence among isolates are identified by the IS*6110* (Haddad *et al*., 2004). In a study by Mulcahy *et al.* (1996), IS*6110*-based PCR was used for the detection of *M. tuberculosis* on 116 isolates. Forty-six of these samples were found positive and the 70 were negative (Mulcahy *et al*., 1996). Spoligotyping is mainly used for the typing of bacteria and the main advantage of this method is the ability to differentiate between *M. bovis* and *M. tuberculosis* (Bolanos *et al*., 2017). Other typing techniques that are used for genotyping of members of MTB are variable number of tandem repeats (VNTR) and DNA sequencing (Bernitz *et al*., 2021). The VNTR analyzes allelic variations that occur repetitively on the genome referred to as tandem repeats (Ghavidel *et al*., 2018). When mycobacteria are being analysed using the VNTR, differences in the repetition of sequences in the genome are analysed (Cole *et al*., 1998; Roring *et al*., 2002). In Uganda, Dickman *et al.,* (2010), from 113 patients that were smear and culture positive, 7.1% of the patients were infected with the multiple strain of *M. tuberculosis* while the 105 were infected with a single strain of *M. tuberculosis*. DNA

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sequencing is also another typing technique that is based on the determination of the exact sequence of nucleotides in the genome (Bolanos *et al*., 2017).

2.17. Diagnosis in humans

2.17.1. Staining

Staining plays a very critical role in the diagnosis of mycobacterial infections. There are two methods of staining that are used the ZN and the Kinyoun (Bernitz *et al*., 2021). The difference between the two is the ZN stain is a hot acid-fast stain while the latter is a cold acid-fast stain. (De Waard & Robledo, 2007).

2.17.2. Chest X-rays

Chest X-rays (CXR) are a valuable tool used for the detection of TB. CXR have been used as a screening tool for the pulmonary TB, especially for patients that are asymptomatic. World Health Organisation recommended that CXR should be used after: a negative bacteriological test, broad-spectrum antibiotics, and a second round of negative bacteriological testing (WHO 2003).

2.17.3. Culture

Culture is still considered the gold standard in the diagnosis of TB. Culture is normally done at first to confirm the diagnosis (Michel *et al*., 2010). A positive culture for *M. tuberculosis* acts as a confirmation of the presence of the disease. Egg-based media such as LJ are used for culturing (De Waard & Robledo, 2007). Since *M. tuberculosis* bacilli have a characteristic of slow growth in culture media, its non-pigmented colonies are only visible after two weeks (Kubica *et al*., 1984). Ziehl-Neelsen staining should be implemented as a confirmatory test for culture-positive mycobacteria and further identification molecular methods should be done (De Waard & Robledo, 2007).

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2.17.4. Tuberculin Skin Test (TST)

The TST has been used mainly for detecting the prevalence of TB in communities. Purified Protein Derivative is normally injected into the patient and a reaction is monitored between 48-72 h (Huebner *et al.*, 1993). A positive reactor will be indicated by the size of the reaction approximately >10 mm and redness. The disadvantage of the test is false positives results may occur as a result of exposure to BCG vaccine and environmental mycobacteria (de Waard & Robledo, 2007).

2.17.5. QuantiFERON –TB test

QuantiFERON-TB in humans is used for the detection of latent *M. tuberculosis* infection. It measures the IFN-γ released by T-cells. The limitations of the test are that blood must be tested very shortly after collection (Caglayan *et al.*, 2011) and also active *M. tuberculosis* may produce a negative result especially for immunocompromised patients (Hornum *et al.*, 2008; Ndzi *et al.*, 2016) check articles write correctly

2.18. Control and Prevention of bovine tuberculosis

The control of bTB is more difficult because of wildlife reservoirs, which might re-infect livestock, particularly in wildlife-livestock interface areas (Caron *et al.*, 2016). The potential transmission of *M. bovis* from wildlife to livestock, particularly cattle, poses a challenge to any control or eradication effort of bTB (Miller, 2015). In South Africa, a study conducted by Musoke *et al.* (2015) revealed a spillover of *M. bovis* infection from wildlife in a game reserve close to cattle and other livestock in neighbouring communal farms. In this country, *M. bovis* has colonised the African buffalo (*Syncerus caffer*), an economically important species, which is part of the 'Big Five' (Lions, Elephants, Rhinoceros, Buffalo, and Leopard) and serves as a wildlife reservoir for bTB (Michel *et al.*, 2010).

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2.18.1. Control of tuberculosis in animals by test and slaughter

In Spain (Naranjo *et al*., 2008) the test and slaughter method is implemented to control TB (Cosivi *et al*., 1998). This has been mainly successful for the reduction of *M. bovis* especially if no other reservoir host of infection is in existence and in areas where the prevalence of TB is low (Dibaba & Kriek, 2019). In most African countries, the cattle and human populations co-exist with no programmes in place for monitoring bTB resulting in the spread of the zoonosis through co-infections between humans and cattle (Cosivi *et al*., 1998).

In Africa, out of the 55 countries, only seven, including South Africa, can adequately apply the test and slaughter method primarily because the costs of control measures are not affordable in most countries. The result, therefore, is that in most African countries, bTB is either partially controlled or not controlled at all. In Asia, out of the 36 nations, only seven countries which apply the test and slaughter method the remaining 29 countries the disease is partly controlled or not controlled at all. Out of the 34 Latin America and Caribbean countries, 12 control bTB by the test and slaughter method and within the remaining 22 countries, the disease is either partially controlled or not controlled (Cosivi *et al*., 1998).

2.18.2. Control of tuberculosis in animals by vaccination

The only feasible solution for protecting both wildlife and livestock against bTB is the use of vaccines, but there is no effective vaccine available to date in South Africa and around the world. *Bacillus* Calmette-Guerin (BCG) is the only vaccine that is used and it only affords partial protection ranging from 0-80% for both animals and humans (Buddle *et al*., 2008). Vaccination strategies to improve the efficacy of BCG for the control of TB are currently being researched including the delivery strategies of the vaccine. For example, oral routes used for delivering inactivated vaccines may not be as effective as live vaccines administered through the duodenum (Buddle *et al*., 2008). Another concept that needs further investigation is to boost the efficacy of the vaccine by investigating whether the vaccine should be given at intervals or

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administered together with other formulations. Another aspect that has complicated the research towards the development of an effective vaccine for TB are variations in study designs, strains used, and doses used for delivery (Waters *et al*., 2012). The effectiveness of the vaccine under field conditions was tested by Lopez-Valencia *et al*. (2010). This proved that the BCG vaccine, even though it did not result in 100% protection, 59% of the vaccinated cattle were protected from getting infected and it was also suggested that the protective efficacy of the BCG vaccine should be tested over a longer study period (Lopez-Valencia *et al*., 2010). Subunit vaccines have been developed to boost the efficacy of the BCG vaccine (Waters *et al*., 2012).

2.18.3. Control of tuberculosis by vaccination and drugs in humans

Vaccination in humans has played a very important role in the control of TB. The BCG (BacilleCalmette-Guerin) vaccine is currently the only licensed vaccine administered to children (Lawn& Zuma, 2011). Protection by this vaccine against TB is reduced 10 years post-vaccination (Trunz *et al*., 2006), thus there is a need for long-term post-exposure vaccination to prevent the reactivation of the bacteria (Lawn & Zumla, 2011). Antimicrobial agents, such as isoniazid and rifampicin, are normally administered to individuals infected with the pathogen. This resulted in the launch of a control strategy called DOTS (directly observed treatment short course) and its main focus is the diagnosis and treatment of TB (Lawn & Zumla, 2011).

2.18.4. Control of tuberculosis in humans by milk pasteurization

Pasteurisation of milk is essential but for consumption in rural communities, this situation is made difficult due to lack of proper infrastructure and facilities for milk processing in rural areas (Ayele *et al*., 2004). Studies have demonstrated that cattle pass the pathogen into milk and pasteurisation, boiling or ultra-high temperature (UHT) treatment of milk is essential before consumption (Bolanos *et al.,* 2017). In Brazil, approximately 30.0% of informal milk is

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sold without inspection and this poses a serious threat as the pathogenic strains of *Mycobacterium* spp. could be transmitted in the process (Motta *et al.*, 2015). In a study conducted by Kazwala *et al.* (1998) in Tanzania where milk was consumed raw, out of 809 milk samples tested from cattle, 3.9% were confirmed to be positive for mycobacteria of which two were *M. bovis* isolates, and the rest of the isolates were identified as NTMs (Kazwala *et al.*, 1998). In SA, according to Dibaba & Kriek (2019) SA and Namibia are currently the only African countries where pasteurised milk is available almost to the entire population, this means pasteurised milk is widely available in South Africa.

2.18.5. Control of tuberculosis by meat inspection at abattoirs

Meat inspection in abattoirs and thorough cooking are also essential in the control of bTB (Ayele *et al.*, 2004). Livestock and wildlife species are slaughtered in abattoirs before the meat is released for human consumption, making them ideal monitoring areas for the screening of carcasses (Kaneene *et al.*, 2006). The most important reason for the examination of carcasses is of public health importance. Information obtained from abattoirs provides useful information on the prevalence of bTB in slaughter animals, and by extension, on the farms where slaughter animals originate. Data obtained from abattoirs also provides insight for policymakers, the risk posed to abattoir workers, surveillance of TB, the geographic spread of the disease, as well as, the opportunity to trace infected herds (Javis & Valdes-Donoso, 2018).

For instance, a study was conducted in different abattoirs in Ri-Bhoi district in Meghalaya, India, to investigate the prevalence of bTB confirmed that 15% of 120 animals tested were positive for the disease (Barua *et al.*, 2016). In another study in Algeria, two abattoirs in Algiers and Blida, 7,250 animals were examined during meat inspection. The study revealed a bTB prevalence of 1.2% (89/7250) (Sahraoui *et al.*, 2009).

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In 2006, a study conducted by Shitaye *et al*. (2006) on cattle, sheep, goats, pigs from an abattoir in Addis Ababa, Ethiopia, during routine meat inspection revealed that 3.5% of the meat was infected with mycobacteria. This proved that abattoir diagnosis for bTB is very crucial, as other diagnostic options are limited in Ethiopia (Shitaye *et al*., 2006).

2.18.6. Control of tuberculosis in animals in South Africa

Several strategies have been implemented in South Africa for the control of TB (Dibaba & Kriek, 2019). Firstly, bovine TB in cattle is considered and listed as a controlled and notifiable disease in terms of the Animal Disease Act 35 of 1984 (Department of Agriculture Forestry and Fisheries, 2013). Notification to the authorities is compulsory in a case of a suspect or identification signalling the presence of the disease. In addition, there are regulations in place that include quarantine, testing schemes, trace, control, and combat programme (Department of Agriculture Forestry and Fisheries, 2016). The South African government has also established a slaughtering out policy for animals that have tested positive for bTB. This policy is under the Tuberculosis Eradication Programme (Department of Agriculture Forestry and Fisheries, 2016). In HiP, a test and slaughter method was implemented in the buffalo population. After an investigation on the disease management by using test and cull in HiP, Le Roex *et al.* (2016), concluded that the method was successful as it resulted in a decrease in the prevalence of bTB. Furthermore, the installation of fences between game parks and livestock, separate the buffaloes and cattle. This reduces the risk of spillover between livestock and wildlife (Department of Agriculture Forestry and Fisheries, 2016).

A study by Michel *et al*., (2008) reported the prevalence of the disease to be 0.4% in 1995 in commercial cattle, while Hlokwe *et al.* (2014) focused on the increase in the spatial distribution of bovine tuberculosis in all the nine provinces of South Africa. However, these studies were not abattoir-based. As such we report on the first abattoir-based study on bTB in slaughter livestock in Gauteng province in SA.

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2.19. Aim

The study aimed to determine the prevalence and characterise *Mycobacterium tuberculosis/M. bovis* and other *Mycobacterium* spp. in slaughter cattle and sheep in abattoirs in Gauteng province, South Africa and to assess the potential health risk posed to abattoir workers.

2.20. Objectives

- To review diagnostic laboratory data (DLD) on the cultures of *Mycobacterium* spp. from livestock and game in South Africa available at the TB laboratory at the ARC-OVR, South Africa between 2007 and 2016.
- To review DLD on serological assays using IFN-γ assay for *Mycobacterium tuberculosis* complex spp. in livestock and game species at the TB laboratories ARC-OVR, South Africa: A 6-year analysis (2011-2016).
- To analyse the knowledge, attitude, and practice (KAP) data from red meat abattoir workers in Gauteng province, South Africa
- To assess the risk of exposure of abattoir workers to *Mycobacterium* spp.
- To determine the prevalence of bovine tuberculosis in slaughter animals in selected abattoirs in Gauteng province, South Africa, using the interferon-gamma assay.
- To isolate, identify and characterize *Mycobacterium* spp. isolated from slaughtered livestock tissues and environmental samples in red meat abattoirs of Gauteng province, South Africa.

2.21. Benefits of the project

Abattoirs play a significant role in passive and active surveillance of diseases because they provide useful information that could serve as early warning systems for future disease outbreaks. In addition, information from abattoirs reveals the status of the disease from the different areas or farms where slaughtered animals originate. Therefore, abattoirs serve as an

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important tool for passive disease surveillance. We hypothesize that the study will provide useful information on the status of tuberculosis in slaughter livestock in Gauteng abattoirs to ensure that contaminated meat does not end up in the retail markets. The study will also assess the potential risk posed to abattoir workers.

2.22. References

Alexander, K.A., Laver, P.N., Michel, A.L., Williams, M., van Helden, P.D., Warren, R.M. and van Pittius, N.C.G., 2010. Novel Mycobacterium tuberculosis complex pathogen, *M. mungi*. *Emerging Infectious Diseases*, *16*(8), p.1296.

Alfredsen, S. and Saxegaard, F., 1992. An outbreak of tuberculosis in pigs and cattle caused by *Mycobacterium africanum*. *The Veterinary Record*, *131*(3), pp.51-53.

Ameni, G. and Erkihun, A., 2007. Bovine tuberculosis on small-scale dairy farms in Adama Town, central Ethiopia, and farmer awareness of the disease. *Revue Scientifique et Technique-Office International des Epizooties*, *26*(3), pp.711-720.

Ameni, G., Hewinson, G., Aseffa, A., Young, D. and Vordermeier, M., 2008. Appraisal of interpretation criteria for the comparative intradermal tuberculin test for diagnosis of tuberculosis in cattle in central Ethiopia. *Clinical and Vaccine Immunology*, *15*(8), pp.1272-1276.

Arega, S.M., Conraths, F.J. and Ameni, G., 2013. Prevalence of tuberculosis in pigs slaughtered at two abattoirs in Ethiopia and molecular characterization of *Mycobacterium tuberculosis* isolated from tuberculous-like lesions in pigs. *BMC Veterinary Research*, *9*(1), pp.1-9.

Awah-Ndukum, J., Kudi, A.C., Bradley, G., Ane-Anyangwe, I., Titanji, V.P.K., Fon-Tebug, S. and Tchoumboue, J., 2012. Prevalence of bovine tuberculosis in cattle in the highlands of

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Cameroon based on the detection of lesions in slaughtered cattle and tuberculin skin tests of live cattle. *Veterinarni Medicina*, *57*(2), p.59.

Ayele, W.Y., Neill, S.D., Zinsstag, J., Weiss, M.G. and Pavlik, I., 2004. Bovine tuberculosis: an old disease but a new threat to Africa. *The International Journal of Tuberculosis and Lung Disease*, *8*(8), pp.924-937.

Barua, A.G. and andChandrani Goswami, H.R., 2016. Slaughter house surveillance for tuberculosis among cattle in Ri-Bhoi district of Meghalaya. *Culture*, *18*, pp.15-0.

Bengis, R. G., Kriek, N. P., Keet, D. F., Raath, J. P., de Vos V. and Huchzermeyer, H. F., 1996. An outbreak of bovine tuberculosis in a free-living African buffalo (*Syncerus caffer--sparrman*) population in the Kruger National Park: a preliminary report. *Onderstepoort Journal of Veterinary Research, 63*(1), pp.15-8.

Biet, F. and Boschiroli, M.L., 2014. Non-tuberculous mycobacterial infections of veterinary relevance. *Research in Veterinary Science*, *97*, pp.S69-S77.

Bercovier, H. and Vincent, V., 2001. Mycobacterial infections in domestic and wild animals due to *Mycobacterium marinum*, *M. fortuitum*, *M. chelonae*, M. porcinum*, M. farcinogenes*, *M. smegmatis*, *M. scrofulaceum*, *M. xenopi*, *M. kansasii*, *M. simiae* and *M. genavense*. *Revue scientifique et technique (International Office of Epizootics)*, *20*(1), pp.265-290.

Bernitz, N., Kerr, T.J., Goosen, W.J., Chileshe, J., Higgitt, R.L., Roos, E.O., Meiring, C., Gumbo, R., de Waal, C., Clarke, C. and Smith, K., 2021. Review of diagnostic tests for detection of *Mycobacterium bovis* infection in South African wildlife. *Frontiers in Veterinary Science*, p.26.

© University of Pretoria

Berrada, J., 1993. *Mycobacterium bovis infection in cattle in Morocco: preparation and evaluation of chemical extracts for use in detection of immune responses* (Doctoral dissertation, Iowa State University).

Berrada, J., 2006. Capacity building for surveillance and control of tuberculosis. *FAO animal production and health proceedings. FAO/WHO/OIE Expert and Technical Consultation, Rome*, pp.49-53.

Bhembe, N.L., Jaja, I.F., Nwodo, U.U., Okoh, A.I. and Green, E., 2017. Prevalence of tuberculous lymphadenitis in slaughtered cattle in Eastern Cape, South Africa. *International Journal of Infectious Diseases*, *61*, pp.27-37.

Biffa, D., Skjerve, E., Oloya, J., Bogale, A., Abebe, F., Dahle, U., Bohlin, J. and Djønne, B., 2010. Molecular characterization of Mycobacterium bovis isolates from Ethiopian cattle. *BMC Veterinary Research*, *6*(1), pp.1-11.

Bilal, S., Iqbal, M., Murphy, P. and Power, J., 2010. Human bovine tuberculosis–remains in the differential. *Journal of Medical Microbiology*, *59*(11), pp.1379-1382.

Bokonyi, S. 1977. Animal remains from Kermanshah valley Iran, BAR supplementary series. Archeolological Science Supplement 34

Bolaños, C.A.D., Paula, C.L.D., Guerra, S.T., Franco, M.M.J. and Ribeiro, M.G., 2017. Diagnosis of mycobacteria in bovine milk: an overview. *Revista do Instituto de Medicina Tropical de São Paulo*, *59*.

Buddle, B.M., Denis, M., Aldwell, F.E., Vordermeier, H.M., Hewinson, R.G. and Wedlock, D.N., 2008. Vaccination of cattle with *Mycobacterium bovis* BCG by a combination of systemic and oral routes. *Tuberculosis*, *88*(6), pp.595-600.

© University of Pretoria

Burke, V. and Barnes, M.W., 1929. The cell wall and the Gram reaction. *Journal of Bacteriology*, *18*(2), pp.69-92.

Cadmus, S.I., Adesokan, H.K., Jenkins, A.O. and Van Soolingen, D., 2009. *Mycobacterium bovis* and M. tuberculosis in goats, Nigeria. *Emerging infectious diseases*, *15*(12), p.2066.

Çağlayan, V., Öznur, A.K., Dabak, G., Damadoğlu, E., Ketenci, B., Özdemir, M., Özer, S. and Saygi, A., 2011. Comparison of tuberculin skin testing and QuantiFERON-TB Gold-In Tube test in health care workers. *Tuberkuloz ve Toraks, 59*(1), p.43-47.

Carneiro, P.A.M., de Moura Sousa, E., Viana, R.B., Monteiro, B.M., Kzam, A.D.S.L., de Souza, D.C., Coelho, A.S., Ribeiro Filho, J.D., Jordao, R.S., Tavares, M.R.M. and Kaneene, J.B., 2021. Study on supplemental test to improve the detection of bovine tuberculosis in individual animals and herds. *BMC Veterinary Research*, *17*(1), pp.1-8.

Caron, A., Cornelis, D., Foggin, C., Hofmeyr, M. and de Garine-Wichatitsky, M., 2016. African buffalo movement and zoonotic disease risk across transfrontier conservation areas, Southern Africa. *Emerging Infectious Diseases*, *22*(2), p.277.

Cassidy, J.P., Bryson, D.G., Pollock, J.M., Evans, R.T., Forster, F. and Neill, S.D., 1998. Early lesion formation in cattle experimentally infected with *Mycobacterium bovis*. *Journal of Comparative Pathology*, *119*(1), pp.27-44.

Cassidy, J.P., Bryson, D.G., Cancela, M.G., Forster, F., Pollock, J.M. and Neill, S.D., 2001. Lymphocyte subtypes in experimentally induced early-stage bovine tuberculous lesions. *Journal of comparative pathology*, *124*(1), pp.46-51.

Chihota, V.N., Grant, A.D., Fielding, K., Ndibongo, B., Van Zyl, A., Muirhead, D. and Churchyard, G.J., 2010. Liquid vs. solid culture for tuberculosis: performance and cost in a

© University of Pretoria

resource-constrained setting. *The international Journal of Tuberculosis and Lung Disease*, *14*(8), pp.1024-1031.

Cole, S., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., Gordon, S.V., Eiglmeier, K., Gas, S., Barry, C.3. and Tekaia, F., 1998. Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. *Nature*, *396*(6707), pp.190-190.

Cooper, A.M., 2009. Cell-mediated immune responses in tuberculosis. *Annual Review of Immunology*, *27*, pp.393-422.

Corner, L.A., 1994. Post mortem diagnosis of *Mycobacterium bovis* infection in cattle. *Veterinary Microbiology*, *40*(1-2), pp.53-63.

Coros, A., DeConno, E. and Derbyshire, K.M., 2008. IS 6110, a Mycobacterium tuberculosis complex-specific insertion sequence, is also present in the genome of *Mycobacterium smegmatis*, suggestive of lateral gene transfer among mycobacterial species. *Journal of Bacteriology*, *190*(9), pp.3408-3410.

Cosivi, O., Grange, J.M., Daborn, C.J., Raviglione, M.C., Fujikura, T., Cousins, D., Robinson, R.A., Huchzermeyer, H.F., de Kantor, I. and Meslin, F.X., 1998. Zoonotic tuberculosis due to *Mycobacterium bovis* in developing countries. *Emerging Infectious Diseases*, *4*(1), p.59.

Cosivi, O., Meslin, F.X., Daborn, C.J. and Grange, J.M., 1995. Epidemiology of *Mycobacterium bovis* infection in animals and humans, with particular reference to Africa. *Revue scientifique et technique (International Office of Epizootics)*, *14*(3), pp.733-746.

© University of Pretoria

Costello, E., Doherty, M.L., Monaghan, M.L., Quigley, F.C. and O'Reilly, P.F., 1998. A study of cattle-to-cattle transmission of *Mycobacterium bovis* infection. *The Veterinary Journal*, *155*(3), pp.245-250.

Courtenay, O., Reilly, L.A., Sweeney, F.P., Hibberd, V., Bryan, S., Ul-Hassan, A., Newman, C., Macdonald, D.W., Delahay, R.J., Wilson, G.J. and Wellington, E.M.H., 2006. Is *Mycobacterium bovis* in the environment important for the persistence of bovine tuberculosis? *Biology letters*, *2*(3), pp.460-462.

Cousins, D.V. and Florisson, N., 2005. A review of tests available for use in the diagnosis of tuberculosis in non-bovine species. *Revue scientifique et technique (International Office of Epizootics)*, *24*(3), pp.1039-1059.

Daffé, M. and Draper, P., 1997. The envelope layers of mycobacteria with reference to their pathogenicity. *Advances in Microbial Physiology*, *39*, pp.131-203.

Dalovisio, J.R., Stetter, M. and Mikota-Wells, S., 1992. Rhinoceros' rhinorrhea: cause of an outbreak of infection due to airborne *Mycobacterium bovis* in zookeepers. *Clinical Infectious Diseases*, *15*(4), pp.598-600.

De la Rua-Domenech, R., Goodchild, A.T., Vordermeier, H.M., Hewinson, R.G., Christiansen, K.H. and Clifton-Hadley, R.S., 2006. Ante mortem diagnosis of tuberculosis in cattle: a review of the tuberculin tests, γ-interferon assay and other ancillary diagnostic techniques. *Research in Veterinary Science*, *81*(2), pp.190-210.

De Lisle, G.W., Bengis, R.G., Schmitt, S.M. and O Brien, D.J., 2002. Tuberculosis in free-ranging wildlife: detection, diagnosis and management. *Revue Scientifique et Technique-Office International des Epizooties*, *21*(1), pp.317-334.

© University of Pretoria

De Vos, V., Bengis, R.G., Kriek, N.P.J., Keet, D.F., Raath, J.P., Huchzermeyer, H.F. and Michel, A.L., 2001. The epidemiology of tuberculosis in free-ranging African buffalo (*Syncerus caffer*) in the Kruger National Park, South Africa. *Onderstepoort Journal of Veterinary Research, 68*(2), pp.119-130.

De Vos, V., McCully, R.M. and Van Niekerk, C.A.W.J., 1977. Mycobacteriosis in the Kruger National Park. *Koedoe*, *20*(1), pp.1-9.

De Waard, J.H. and Robledo, J., 2007. Conventional diagnostic methods. *Tuberculosis*, pp.401-424.

Department of Agriculture, Forestry and Fisheries (DAFF), Republic of South Africa.2013. Interim bovine tuberculosis scheme manual. (6 December 2013). http://ww.nda.agric.agric.za/vetweb/pamphlets&Information/Policy/TB

Department of Agriculture, Forestry and Fisheries (DAFF), Republic of South Africa.2016. Interim bovine tuberculosis scheme manual. (20 September 2016). http://ww.nda.agric.agric.za/vetweb/pamphlets&Information/Policy/TB

Dhama, K., Mahendran, M., Tiwari, R., Dayal Singh, S., Kumar, D., Singh, S. and Sawant, P.M., 2011. Tuberculosis in birds: insights into the *Mycobacterium avium* infections. *Veterinary Medicine International*, *2011*.

Dibaba, A.B. and Kriek, N.P., 2019. The Control of Bovine Tuberculosis in Africa. In *Tuberculosis in Animals: An African Perspective* (pp. 237-270). Springer, Cham.

Dickman, K.R., Nabyonga, L., Kateete, D.P., Katabazi, F.A., Asiimwe, B.B., Mayanja, H.K., Okwera, A., Whalen, C. and Joloba, M.L., 2010. Detection of multiple strains of *Mycobacterium tuberculosis* using MIRU-VNTR in patients with pulmonary tuberculosis in Kampala, Uganda. *BMC infectious diseases*, *10*(1), pp.1-7.

© University of Pretoria

Diguimbaye-Djaibé, C., Vincent, V., Schelling, E., Hilty, M., Ngandolo, R., Mahamat, H.H., Pfyffer, G., Baggi, F., Tanner, M. and Zinsstag, J., 2006. Species identification of non-tuberculous mycobacteria from humans and cattle of Chad. *Schweizer Archiv für Tierheilkunde*, *148*(5), pp.251-256.

Dippenaar, A., Parsons, S.D.C., Miller, M.A., Hlokwe, T., van Pittius, N.C.G., Adroub, S.A., Abdallah, A.M., Pain, A., Warren, R.M., Michel, A.L. and Van Helden, P.D., 2017. Progenitor strain introduction of *Mycobacterium bovis* at the wildlife-livestock interface can lead to clonal expansion of the disease in a single ecosystem. *Infection, Genetics, and Evolution*, *51*, pp.235-238.

Duffy, S.C., Srinivasan, S., Schilling, M.A., Stuber, T., Danchuk, S.N., Michael, J.S., Venkatesan, M., Bansal, N., Maan, S., Jindal, N. and Chaudhary, D., 2020. Reconsidering Mycobacterium bovis as a proxy for zoonotic tuberculosis: a molecular epidemiological surveillance study. *The Lancet Microbe*, *1*(2), pp.e66-e73.

Ejeh, E.F., Raji, M.A., Bello, M., Lawan, F.A., Francis, M.I., Kudi, A.C. and Cadmus, S.I.B., 2014. Prevalence and direct economic losses from bovine tuberculosis in Makurdi, Nigeria. *Veterinary Medicine International*, *2014*, pp.1-6

Kedir, E., Mamo, G., Legesse, K. and Tassew, A., 2018. Review on advanced diagnostic techniques for *Mycobacterium* species and its significance to control tuberculosis. *J. Bacteriol. Mycol. Open Access*, *6*(3), pp.168-177.

Feizabadi, M.M., Robertson, I.D., Cousins, D.V. and Hampson, D.J., 1996. Genomic analysis of *Mycobacterium bovis* and other members of the Mycobacterium tuberculosis complex by isoenzyme analysis and pulsed-field gel electrophoresis. *Journal of Clinical Microbiology*, *34*(5), pp.1136-1142.

© University of Pretoria

Feldman, M., Isaza, R., Prins, C. and Hernandez, J., 2013. Point prevalence and incidence of *Mycobacterium tuberculosis* complex in captive elephants in the United States of America. *Veterinary Quarterly*, *33*(1), pp.25-29.

Gcebe, N. and Hlokwe, T.M., 2017. Non-tuberculous mycobacteria in South African wildlife: neglected pathogens and potential impediments for bovine tuberculosis diagnosis. *Frontiers in Cellular and Infection Microbiology*, *7*, p.15.

Gcebe, N., Michel, A., Gey van Pittius, N.C. and Rutten, V., 2016. Comparative genomics and proteomic analysis of four non-tuberculous *Mycobacterium* species and Mycobacterium tuberculosis complex: occurrence of shared immunogenic proteins. *Frontiers in Microbiology*, *7*, p.795.

Gcebe, N., Rutten, V., Gey van Pittius, N.C. and Michel, A., 2013. Prevalence and Distribution of Non-Tuberculous Mycobacteria (NTM) in Cattle, African Buffaloes (*S yncerus caffer*) and their Environments in South Africa. *Transboundary and Emerging Diseases*, *60*, pp.74-84.

Ghavidel, M., Mansury, D., Nourian, K. and Ghazvini, K., 2018. The most common spoligotype of *Mycobacterium bovis* isolated in the world and the recommended loci for VNTR typing; A systematic review. *Microbial Pathogenesis*, *118*, pp.310-315.

Gormley, E., Corner, L.A.L., Costello, E. and Rodriguez-Campos, S., 2014. Bacteriological diagnosis and molecular strain typing of *Mycobacterium bovis* and *Mycobacterium caprae. Research in Veterinary Science*, *97*, pp.S30-S43.

Haddad, N., Masselot, M. and Durand, B., 2004. Molecular differentiation of *Mycobacterium bovis* isolates. Review of main techniques and applications. *Research in Veterinary Science*, *76*(1), pp.1-18.

© University of Pretoria

Hlavsa, M.C., Moonan, P.K., Cowan, L.S., Navin, T.R., Kammerer, J.S., Morlock, G.P., Crawford, J.T., and LoBue, P.A., 2008. Human tuberculosis due to Mycobacterium bovis in the United States, 1995-2005. *Clinical Infectious Diseases*, *47*(2), pp.168-175.

Hlokwe, T.M., Van Helden, P. and Michel, A.L., 2014. Evidence of increasing intra and inter-species transmission of *Mycobacterium bovis* in South Africa: are we losing the battle? *Preventive Veterinary Medicine*, *115*(1-2), pp.10-17.

Hlokwe, T.M., De Klerk-Lorist, L.M. and Michel, A.L., 2016. Wildlife on the move: a hidden tuberculosis threat to conservation areas and game farms through introduction of untested animals. *Journal of Wildlife Diseases*, *52*(4), pp.837-843.

Hlokwe, T.M., Said, H. and Gcebe, N., 2017. *Mycobacterium tuberculosis* infection in cattle from the Eastern Cape Province of South Africa. *BMC veterinary research*, *13*(1), pp.1-9.

Hlokwe, T.M., Michel, A.L., Mitchel, E., Gcebe, N. and Reininghaus, B., 2019. First detection of *Mycobacterium bovis* infection in Giraffe (*Giraffa camelopardalis*) in the Greater Kruger National Park Complex: Role and implications. *Transboundary and Emerging Diseases*, *66*(6), pp.2264-2270.

Hlokwe, T.M. and Mogano, R.M., 2020. Utility of xpert® MTB/RIF ultra assay in the rapid diagnosis of bovine tuberculosis in wildlife and livestock animals from South Africa. *Preventive Veterinary Medicine*, *177*, p.104980

Hornum, M., Mortensen, K.L., Kamper, A.L. and Andersen, Å.B., 2008. Limitations of the QuantiFERON®-TB Gold test in detecting Mycobacterium tuberculosis infection in immunocompromised patients. *European journal of internal medicine*, *19*(2), pp.137-139.

Huebner, R.E., Schein, M.F. and Bass Jr, J.B., 1993. The tuberculin skin test. *Clinical Infectious Diseases*, pp.968-975.

© University of Pretoria

Hutcheon, D., 1880. Tering, consumption, tables mesenterica. *Annual Report, Colonial Veterinary Surgeon, Cape of Good Hope*.

Johnson, M.M. and Odell, J.A., 2014. Nontuberculous mycobacterial pulmonary infections. *Journal of thoracic disease*, *6*(3), p.210.

Liebana, E., Johnson, L., Gough, J., Durr, P., Jahans, K., Clifton-Hadley, R., Spencer, Y., Hewinson, R.G. and Downs, S.H., 2008. Pathology of naturally occurring bovine tuberculosis in England and Wales. *The Veterinary Journal*, *176*(3), pp.354-360.

Jarvis, L.S. and Valdes-Donoso, P., 2018. A selective review of the economic analysis of animal health management. *Journal of Agricultural Economics*, *69*(1), pp.201-225.

Jolles, A.E., 2004. *Disease ecology of tuberculosis in African buffalo*. Princeton University. PhD Thesis

Jolley, M.E., Nasir, M.S., Surujballi, O.P., Romanowska, A., Renteria, T.B., De la Mora, A., Lim, A., Bolin, S.R., Michel, A.L., Kostovic, M. and Corrigan, E.C., 2007. Fluorescence polarization assay for the detection of antibodies to *Mycobacterium bovis* in bovine sera. *Veterinary Microbiology*, *120*(1-2), pp.113-121.

Kahla, I.B., Boschiroli, M.L., Souissi, F., Cherif, N., Benzarti, M., Boukadida, J. and Hammami, S., 2011. Isolation and molecular characterisation of *Mycobacterium bovis* from raw milk in Tunisia. *African Health Sciences*, *11*, pp.2-5.

Kankya, C., Muwonge, A., Djønne, B., Munyeme, M., Opuda-Asibo, J., Skjerve, E., Oloya, J., Edvardsen, V. and Johansen, T.B., 2011. Isolation of non-tuberculous mycobacteria from pastoral ecosystems of Uganda: public health significance. *BMC Public Health*, *11*(1), pp.1-9.

© University of Pretoria

Kaneene, J.B., Miller, R. and Meyer, R.M., 2006. Abattoir surveillance: the US experience. *Veterinary Microbiology*, *112*(2-4), pp.273-282.

Katale, B.Z., Mbugi, E.V., Botha, L., Keyyu, J.D., Kendall, S., Dockrell, H.M., Michel, A.L., Kazwala, R.R., Rweyemamu, M.M., Van Helden, P. and Matee, M.I., 2014. Species diversity of non-tuberculous mycobacteria isolated from humans, livestock and wildlife in the Serengeti ecosystem, Tanzania. *BMC Infectious Diseases*, *14*(1), pp.1-8.

Kazwala, R.R., Daborn, C.J., Kusiluka, L.J.M., Jiwa, S.F.H., Sharp, J.M. and Kambarage, D.M., 1998. Isolation of *Mycobacterium* species from raw milk of pastoral cattle of the Southern Highlands of Tanzania. *Tropical Animal Health and Production*, *30*(4), pp.233-239.

Keet, D.F., Kriek, N.P.J., Bengis, R.G. and Michel, A.L., 2001. Tuberculosis in kudus (*Tragelaphus strepsiceros*) in the Kruger National Park. *Onderstepoort Journal of Veterinary Research, 68,* 225-230.

Khattak, I., Mushtaq, M.H., Ahmad, M.U.D., Khan, M.S. and Haider, J., 2016. Zoonotic tuberculosis in occupationally exposed groups in Pakistan. *Occupational Medicine*, *66*(5), pp.371-376.

Koch, R., 1982. The etiology of tuberculosis. *Reviews of infectious diseases*, *4*(6), pp.1270-1274.

Komijn, R.E., de Haas, P.E., Schneider, M.M., Eger, T., Nieuwenhuijs, J.H., van den Hoek, R.J., Bakker, D., van Zijderveld, F.G. and van Soolingen, D., 1999. Prevalence of *Mycobacterium avium* in slaughter pigs in The Netherlands and comparison of IS 1245 restriction fragment length polymorphism patterns of porcine and human isolates. *Journal of Clinical Microbiology*, *37*(5), pp.1254-1259.

© University of Pretoria

Kriek, N.P., Areda, D.B. and Dibaba, A.B., 2019. The diagnosis of bovine tuberculosis. In *Tuberculosis in animals: An African perspective* (pp. 171-235). Springer, Cham.

Lawn, S.D. and Zumla, A.I., 2011. Seminar tuberculosis. *Lancet*, *378*(9785), pp.57-72.

Lekko, Y.M., Ooi, P.T., Omar, S., Mazlan, M., Ramanoon, S.Z., Jasni, S., Jesse, F.F.A. and Che-Amat, A., 2020. Mycobacterium tuberculosis complex in wildlife: Review of current applications of antemortem and postmortem diagnosis. *Veterinary World*, *13*(9), p.1822.

Le Roex, N., Cooper, D., van Helden, P.D., Hoal, E.G. and Jolles, A.E., 2016. Disease control in wildlife: evaluating a test and cull programme for bovine tuberculosis in African buffalo. *Transboundary and Emerging Diseases*, *63*(6), pp.647-657.

Lees, V.W., Copeland, S. and Rousseau, P., 2003. Manitoba: Bovine tuberculosis in elk (*Cervus elaphus manitobensis*) near Riding Mountain National Park, Manitoba, from 1992 to 2002. *The Canadian Veterinary Journal*, *44*(10), p.830.

LoBue, P.A., Enarson, D.A. and Thoen, C.O., 2010. Tuberculosis in humans and animals: an overview [Serialised article. Tuberculosis: a re-emerging disease in animals and humans. Number 1 in the series]. *The International Journal of Tuberculosis and Lung Disease*, *14*(9), pp.1075-1078.

Lopez-Valencia, G., Renteria-Evangelista, T., de Jesus Williams, Licea-Avarro, A., de la Mora-Valle A. & Medina-Basulto. 2010. Field evaluation of the protective efficacy of *Mycobacterium bovis* BGC vaccine against bovine tuberculosis. *Research in Veterinary Science 88* (1) :44-49.

Luboya, L.W., Malangu, M., Kaleka, M., Ngulu, N., Nkokele, B., Maryabo, K., Pourrut, X., Vincent, T., and Gonzalez, J.P., 2017. An assessment of caprine tuberculosis prevalence in

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Lubumbashi slaughterhouse, Democratic Republic of Congo. *Tropical Animal Health and Production*, *49*(4), pp.875-878.

Malama, S., Muma, J.B. and Godfroid, J., 2013. A review of tuberculosis at the wildlife-livestock-human interface in Zambia. *Infectious Diseases of Poverty*, *2*(1), pp.1-5.

Marassi, C.D., Medeiros, L., McNair, J. and Lilenbaum, W., 2011. Use of recombinant proteins MPB70 or MPB83 as capture antigens in ELISAs to confirm bovine tuberculosis infections in Brazil. *Acta Tropica*, *118*(2), pp.101-104.

McIlroy, S.G., Neill, S.D., and McCracken, R.M., 1986. Pulmonary lesions and *Mycobacterium bovis* excretion from the respiratory tract of tuberculin reacting cattle. *The Veterinary Record*, *118*(26), pp.718-721.

Michalak, K., Austin, C., Diesel, S., Bacon, M.J., Zimmerman, P. and Maslow, J.N., 1998. Mycobacterium tuberculosis infection as a zoonotic disease: transmission between humans and elephants. *Emerging Infectious Diseases*, *4*(2), p.283.

Michel, A.L. and Huchzermeyer, H.F.A.K., 1998. The zoonotic importance of *Mycobacterium tuberculosis*: transmission from human to monkey. *Journal of the South African Veterinary Association*, *69*(2), pp.64-65.

Michel, A.L., Venter, L., Espie, I.W. and Coetzee, M.L., 2003. *Mycobacterium tuberculosis* infections in eight species at the National Zoological Gardens of South Africa, 1991–2001. *Journal of Zoo and Wildlife Medicine*, *34*(4), pp.364-370.

Michel, A.L., Bengis, R.G., Keet, D.F., Hofmeyr, M., De Klerk, L.M., Cross, P.C., Jolles, A.E., Cooper, D., Whyte, I.J., Buss, P. and Godfroid, J., 2006. Wildlife tuberculosis in South African conservation areas: implications and challenges. *Veterinary Microbiology*, *112*(2-4), pp.91-100.

© University of Pretoria

Michel, A.L., 2008. *Mycobacterium fortuitum* infection interference with *Mycobacterium bovis* diagnostics: natural infection cases and a pilot experimental infection. *Journal of Veterinary Diagnostic Investigation*, *20*(4), pp.501-503.

Michel, A.L., Müller, B. and Van Helden, P.D., 2010. *Mycobacterium bovis* at the animal–human interface: A problem, or not? *Veterinary Microbiology*, *140*(3-4), pp.371-381.

Michel, A.L., Cooper, D., Jooste, J., De Klerk, L.M. and Jolles, A., 2011. Approaches towards optimising the gamma interferon assay for diagnosing *Mycobacterium bovis* infection in African buffalo (*Syncerus caffer*). *Preventive Veterinary Medicine*, *98*(2-3), pp.142-151.

Michel, A.L., Hlokwe, T.M., Espie, I.W., van Zijll Langhout, M., Koeppel, K. and Lane, E., 2013. *Mycobacterium tuberculosis* at the Human/Wildlife Interface in a High TB Burden Country. *Transboundary and Emerging Diseases*, *60*, pp.46-52.

Michel, A.L., Sibanda, D.R. and de Klerk-Lorist, L.M., 2019. BTB Control Strategies in Livestock and Wildlife in South Africa. In *Tuberculosis in animals: An African perspective* (pp. 387-401). Springer, Cham.

Mikota, S.K., 2008. Tuberculosis in elephants. In *Zoo and Wild Animal Medicine* (pp. 355-364). WB Saunders.

Miller, M. and Olea-Popelka, F., 2013. One Health in the shrinking world: Experiences with tuberculosis at the human–livestock–wildlife interface. *Comparative Immunology, Microbiology, and Infectious Diseases*, *36*(3), pp.263-268.

Miller, M., White, P.C. and Bengis, R.G., 2015. Tuberculosis in South African wildlife: why is it important. *Sun Media: Stellenbosch, South Africa*.

© University of Pretoria

Miller, M.A., Buss, P., Roos, E.O., Hausler, G., Dippenaar, A., Mitchell, E., Van Schalkwyk, L., Robbe-Austerman, S., Waters, W., Sikar-Gang, A. and Lyashchenko, K.P., 2019. Fatal tuberculosis in a free-ranging African elephant and one health implications of human pathogens in wildlife. *Frontiers in Veterinary Science*, *6*, p.18.

Mirsaeidi, M., Farshidpour, M., Allen, M.B., Ebrahimi, G. and Falkinham, J.O., 2014. Highlight on advances in nontuberculous mycobacterial disease in North America. *BioMed Research International*, *2014*.

Mohammed, S., Saidu, A.S., Jajere, S.M., Tomar, P., Wakil, A.M., Mohammed, J. and Preeti, R., 2019. Risk factors assessment of bovine tuberculosis among abattoir personnel in Gombe State, Northeastern Nigeria: A One-Health approach. *International Journal of One Health*, *5*, pp.1-8.

Moiane, I., Machado, A., Santos, N., Nhambir, A., Inlamea, O., Hattendorf, J., Källenius, G., Zinsstag, J. and Correia-Neves, M., 2014. Prevalence of bovine tuberculosis and risk factor assessment in cattle in rural livestock areas of Govuro District in the Southeast of Mozambique. *PloS One*, *9*(3), p.e91527.

Monaghan, M. L., Doherty, M. L., Collins, J. D., Kazda, J. F. & Quinn, P. J. 1994. The tuberculin skin test. *Veterinary Microbiology 40*(1), pp. 111-124.

Montali, R.J., Mikota, S.K. and Cheng, L.I., 2001. Mycobacterium tuberculosis in zoo and wildlife species. *Scientific and Technical Review, International Office of Epizootics* 20(1), pp. 291-303.

Mote, R.F., Muhm, R.L. and Gigstad, D.C., 1975. A Staining Method Using Acridine Orange and Auramine O for Fungi and Mycobacteru in Bovine Tissue. *Stain technology*, *50*(1), pp.5-9.

© University of Pretoria

Motta, R.G., Silva, A.V., Giuffrida, R., Siqueira, A.K., Paes, A.C., Motta, I.G., Listoni, F.J. and Ribeiro, M.G., 2015. Indicators of quality and composition of informal milk marketed in the in the Southeast region of São Paulo, Brazil. *Pesquisa Veterinária Brasileira*, *35*, pp.417-423.

Mulcahy, G.M., Kaminski, Z.C., Albanese, E.A., Sood, R. and Pierce, M., 1996. IS6110-based PCR methods for detection of *Mycobacterium tuberculosis*. *Journal of Clinical Microbiology*, *34*(5), pp.1348-1349.

Müller, B., Steiner, B., Bonfoh, B., Fané, A., Smith, N.H. and Zinsstag, J., 2008. Molecular characterisation of *Mycobacterium bovis* isolated from cattle slaughtered at the Bamako abattoir in Mali. *BMC Veterinary Research*, *4*(1), pp.1-6.

Munyeme, M., Muma, J.B., Samui, K.L., Skjerve, E., Nambota, A.M., Phiri, I.G.K., Rigouts, L. and Tryland, M., 2009. Prevalence of bovine tuberculosis and animal level risk factors for indigenous cattle under different grazing strategies in the livestock/wildlife interface areas of Zambia. *Tropical Animal Health and Production*, *41*(3), pp.345-352.

Munyeme, M., Rigouts, L., Shamputa, I.C., Muma, J.B., Tryland, M., Skjerve, E. and Djønne, B., 2009. Isolation and characterization of *Mycobacterium bovis* strain from indigenous Zambian cattle using Spacer oligonucleotide typing technique. *BMC Microbiology*, *9*(1), pp.1-8.

Musoke, J., Hlokwe, T., Marcotty, T., Du Plessis, B.J. and Michel, A.L., 2015. Spillover of *Mycobacterium bovis* from wildlife to livestock, South Africa. *Emerging infectious diseases*, *21*(3), p.448.

Muwonge, A., Kankya, C., Godfroid, J., Djonne, B., Opuda-Asibo, J., Biffa, D., Ayanaw, T., Munyeme, M. and Skjerve, E., 2010. Prevalence and associated risk factors of mycobacterial

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infections in slaughter pigs from Mubende district in Uganda. *Tropical Animal Health and Production*, *42*(5), pp.905-913.

Neill, S.D., O'Brien, J.J. and Hanna, J., 1991. A mathematical model for *Mycobacterium bovis* excretion from tuberculous cattle. *Veterinary microbiology*, *28*(1), pp.103-109.

Neill, S.D., Hanna, J., Mackie, D.P. and Bryson, T.G., 1992. Isolation of Mycobacterium bovis from the respiratory tracts of skin test-negative cattle. *The Veterinary Record*, *131*(3), pp.45-47.

Naranjo, V., Gortazar, C., Vicente, J. and de la Fuente, J., 2008. Evidence of the role of European wild boar as a reservoir of Mycobacterium tuberculosis complex. *Veterinary Microbiology*, *127*(1-2), pp.1-9.

Ndzi, E.N., Nkenfou, C.N., Gwom, L.C., Fainguem, N., Fokam, J. and Pefura, Y., 2016. The pros and cons of the QuantiFERON test for the diagnosis of tuberculosis, prediction of disease progression, and treatment monitoring. *International Journal of Mycobacteriology*, *5*(2), pp.177-184.

Nugent, G., Buddle, B.M. and Knowles, G., 2015. Epidemiology and control of *Mycobacterium bovis* infection in brushtail possums (*Trichosurus vulpecula*), the primary wildlife host of bovine tuberculosis in New Zealand. *New Zealand Veterinary Journal*, *63*(sup1), pp.28-41.

O'Brien, D.J., Schmitt, S.M., Fitzgerald, S. D. and Berry, D.E., 2011. Management of bovine tuberculosis in Michigan wildlife: current status and near term prospects. *Veterinary Microbiology*, *151*(1-2), pp.179-187.

Paine, R. and Martinaglia, G., 1929. Tuberculosis in wild buck living under natural conditions. *Journal of Comparative Pathology and Therapeutics*, *42*, pp.1-8.

© University of Pretoria

Parsons, L.M., Brosch, R., Cole, S.T., Somoskövi, A., Loder, A., Bretzel, G., Van Soolingen, D., Hale, Y.M. and Salfinger, M., 2002. Rapid and simple approach for identification of *Mycobacterium tuberculosis* complex isolates by PCR-based genomic deletion analysis. *Journal of Clinical Microbiology*, *40*(7), pp.2339-2345.

Parsons, S.D., Drewe, J.A., van Pittius, N.C.G., Warren, R.M. and Van Helden, P.D., 2013. Novel cause of tuberculosis in meerkats, South Africa. *Emerging Infectious Diseases*, *19*(12), p.2004.

Phillips, C.J.C., Foster, C.R.W., Morris, P.A. and Teverson, R., 2003. The transmission of *Mycobacterium bovis* infection to cattle. *Research in veterinary science*, *74*(1), pp.1-15.

Proaño-Pérez, F., Rigouts, L., Brandt, J., Dorny, P., Ron, J., Chavez, M.A., Rodriguez, R., Fissette, K., Van Aerde, A., Portaels, F. and Benitez-Ortiz, W., 2006. Preliminary observations on *Mycobacterium* spp. in dairy cattle in Ecuador. *The American Journal of Tropical Medicine and Hygiene*, *75*(2), pp.318-323.

Perry, B. and Grace, D., 2009. The impacts of livestock diseases and their control on growth and development processes that are pro-poor. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1530), pp.2643-2655.

Pollock, J.M., Pollock, D.A., Campbell, D.G., Girvin, R.M., Crockard, A.D., Neill, S.D. and Mackie, D.P., 1996. Dynamic changes in circulating and antigen-responsive T-cell subpopulations post-Mycobacterium bovis infection in cattle. *Immunology*, *87*(2), pp.236-241.

Pollock, J.M. and Andersen, P., 1997. The potential of the ESAT-6 antigen secreted by virulent mycobacteria for specific diagnosis of tuberculosis. *Journal of Infectious Diseases*, *175*(5), pp.1251-1254.

© University of Pretoria

Pollock, J.M. and Neill, S.D., 2002. *Mycobacterium bovis infection* and tuberculosis in cattle. *The Veterinary Journal*, *163*(2), pp.115-127.

Quinn, P.J., 1994. The Actinomycetes In: Quinn PJ, Carter ME, Markey B, Carter JR Clinical Veterinary Microbiology, Mosby Year book, St.

Renwick, A.R., White, P.C.L. and Bengis, R.G., 2007. Bovine tuberculosis in southern African wildlife: a multi-species host-pathogen system. *Epidemiology & Infection*, *135*(4), pp.529-540.

Rodríguez, S., Bezos, J., Romero, B., de Juan, L., Álvarez, J., Castellanos, E., Moya, N., Lozano, F., Javed, M.T., Sáez-Llorente, J.L. and Liébana, E., 2011. Mycobacterium caprae infection in livestock and wildlife, Spain. *Emerging infectious diseases*, *17*(3), p.532.

Rogall, T., Wolters, J., Flohr, T. and Bottger, E.C., 1990. Towards a phylogeny and definition of species at the molecular level within the genus *Mycobacterium*. *International Journal of Systematic and Evolutionary Microbiology*, *40*(4), pp.323-330.

Roug, A., Perez, A., Mazet, J.A., Clifford, D.L., VanWormer, E., Paul, G., Kazwala, R.R. and Smith, W.A., 2014. Comparison of intervention methods for reducing human exposure to Mycobacterium bovis through milk in pastoralist households of Tanzania. *Preventive Veterinary Medicine*, *115*(3-4), pp.157-165.

Roos, E.O., Buss, P., de Klerk-Lorist, L.M., Hewlett, J., Hausler, G.A., Rossouw, L., McCall, A.J., Cooper, D., van Helden, P.D., Parsons, S.D. and Miller, M.A., 2016. Test performance of three serological assays for the detection of *Mycobacterium bovis* infection in common warthogs (*Phacochoerus africanus*). *Veterinary Immunology and Immunopathology*, *182*, pp.79-84.

© University of Pretoria

Roos, E.O., 2018. *Detection and characterization of mycobacterial infections occurring in Phacochoerus africanus (Gmelin, 1788)(*Common Warthog*)* (Doctoral dissertation, Stellenbosch: Stellenbosch University).

Rothel, J.S., Corner, L.A., Wood, P.R., Jones, S.L. and Cox, J.C., 1990. A sandwich enzyme immunoassay for bovine interferon-gamma and its use for the detection of tuberculosis in cattle [Mycobacterium bovis]. *Australian Veterinary Journal (Australia).*

Roring, S., Scott, A., Brittain, D., Walker, I., Hewinson, G., Neill, S. and Skuce, R., 2002. Development of variable-number tandem repeat typing of Mycobacterium bovis: comparison of results with those obtained by using existing exact tandem repeats and spoligotyping. *Journal of Clinical Microbiology*, *40*(6), pp.2126-2133.

Ryan, T.J., Livingstone, P.G., Ramsey, D.S.L., De Lisle, G.W., Nugent, G., Collins, D.M. and Buddle, B.M., 2006. Advances in understanding disease epidemiology and implications for control and eradication of tuberculosis in livestock: the experience from New Zealand. *Veterinary Microbiology*, *112*(2-4), pp.211-219.

Sahraoui, N., Müller, B., Guetarni, D., Boulahbal, F., Yala, D., Ouzrout, R., Berg, S., Smith, N.H. and Zinsstag, J., 2009. Molecular characterization of *Mycobacterium bovis* strains isolated from cattle slaughtered at two abattoirs in Algeria. *BMC Veterinary Research*, *5*(1), pp. 1-7.

Schiller, I., Oesch, B., Vordermeier, H.M., Palmer, M.V., Harris, B.N., Orloski, K.A., Buddle, B.M., Thacker, T.C., Lyashchenko, K.P. and Waters, W.R., 2010. Bovine tuberculosis: a review of current and emerging diagnostic techniques in view of their relevance for disease control and eradication. *Transboundary and Emerging Diseases*, *57*(4), pp.205-220.

Sherratt, A., 1981. Plough and pastoralism: aspects of the secondary products revolution. *Pattern of the past: studies in honour of David Clarke*, *261*, p.305.

© University of Pretoria

Sichewo, P.R., Hlokwe, T.M., Etter, E. and Michel, A.L., 2020. Tracing cross species transmission of *Mycobacterium bovis* at the wildlife/livestock interface in South Africa. *BMC Microbiology*, *20*(1), pp.1-9.

Shitaye, J.E., Getahun, B., Alemayehu, T., Skoric, M., Treml, F., Fictum, P., Vrbas, V. and Pavlik, I., 2006. A prevalence study of bovine tuberculosis by using abattoir meat inspection and tuberculin skin testing data, histopathological and IS6110 PCR examination of tissues with tuberculous lesions in cattle in Ethiopia. *Veterinary Medicina-Praha*, *51*(11), pp.512.

Silva, D.A.V.D., Siconelli, M.J.L., Bürger, K.P. and Keid, L.B., 2018. Comparison between tests for tuberculosis diagnosis in slaughtered bovines. *Arquivos do Instituto Biológico*, *85,* 1-8.

Skuce, R.A. and Neill, S.D., 2001. Molecular epidemiology of *Mycobacterium bovis*: exploiting molecular data. *Tuberculosis*, *81*(1-2), pp.169-175.

Smith, I., 2003. *Mycobacterium tuberculosis* pathogenesis and molecular determinants of virulence. *Clinical microbiology Reviews*, *16*(3), pp.463-496.

Smithwick, R.W., Bigbie Jr, M.R., Ferguson, R.B., Karlix, M.A. and Wallis, C.K., 1995. Phenolic acridine orange fluorescent stain for mycobacteria. *Journal of clinical microbiology*, *33*(10), pp.2763-2764.

Snider Jr, D.E., 1982. The tuberculin skin test. *American Review of Respiratory Disease*, *125*(3P2), pp.108-118.

Sunder, S., Lanotte, P., Godreuil, S., Martin, C., Boschiroli, M.L. and Besnier, J.M., 2009. Human-to-human transmission of tuberculosis caused by *Mycobacterium bovis* in immunocompetent patients. *Journal of Clinical Microbiology*, *47*(4), pp.1249-1251.

© University of Pretoria

Taylor, G.M., Murphy, E., Hopkins, R., Rutland, P. and Chistov, Y., 2007. First report of *Mycobacterium bovis* DNA in human remains from the Iron Age. *Microbiology*, *153*(4), pp.1243-1249.

Thoen, C., LoBue, P. and De Kantor, I., 2006. The importance of Mycobacterium bovis as a zoonosis. *Veterinary Microbiology*, *112*(2-4), pp.339-345.

Theon, C. O., Karlson, A. G. and Himes, E. M. 1981. Mycobacterial Infections in animals. *Reviews of Infectious Diseases 3*(5), pp. 960-971.

Thierry, D., Brisson-Noël, A., Vincent-Lévy-Frébault, V., Nguyen, S., Guesdon, J.L. and Gicquel, B., 1990. Characterization of a *Mycobacterium tuberculosis* insertion sequence, IS6110, and its application in diagnosis. *Journal of Clinical Microbiology*, *28*(12), pp.2668-2673.

Thoen, C.O., LoBue, P.A., Enarson, D.A., Kaneene, J.B. and de Kantor, I.N., 2009. Tuberculosis: a re-emerging disease in animals and humans. *Veterinaria Italiana, 45*(1), pp.35-81.

Trunz, B.B., Fine, P.E.M. and Dye, C., 2006. Effect of BCG vaccination on childhood tuberculous meningitis and miliary tuberculosis worldwide: a meta-analysis and assessment of cost-effectiveness. *The Lancet*, *367*(9517), pp.1173-1180.

Van Embden, J.D., Cave, M.D., Crawford, J.T., Dale, J.W., Eisenach, K.D., Gicquel, B., Hermans, P., Martin, C., McAdam, R. and Shinnick, T.M., 1993. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. *Journal of Clinical Microbiology*, *31*(2), pp.406-409.

Varello, K., Pezzolato, M., Mascarino, D., Ingravalle, F., Caramelli, M. and Bozzetta, E., 2008. Comparison of histologic techniques for the diagnosis of bovine tuberculosis in the

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framework of eradication programs. *Journal of Veterinary Diagnostic Investigation*, *20*(2), pp.164-169.

van Pittius, N.C.G., Gamieldien, J., Hide, W., Brown, G.D., Siezen, R.J. and Beyers, A.D., 2001. The ESAT-6 gene cluster of *Mycobacterium tuberculosis* and other high G+ C Gram-positive bacteria. *Genome Biology*, *2*(10), pp.1-18.

van Pittius, N.C.G.V., Perrett, K.D., Michel, A.L., Keet, D.F., Hlokwe, T., Streicher, E.M., Warren, R.M. and van Helden, P.D., 2012. Infection of African buffalo (*Syncerus caffer*) by oryx bacillus, a rare member of the antelope clade of the Mycobacterium tuberculosis complex. *Journal of Wildlife Diseases*, *48*(4), pp.849-857.

Vekemans, M., Cartoux, M., Diagbouga, S., Dembélé, M., Koné, B., Delafosse, A., Dera, A. and Van de Perre, P., 1999. Potential source of human exposure to Mycobacterium bovis in Burkina Faso, in the context of the HIV epidemic. *Clinical Microbiology and Infection*, *5*(10), pp.617-621.

Vordermeier, H.M., Brown, J., Cockle, P.J., Franken, W.P., Arend, S.M., Ottenhoff, T.H., Jahans, K. and Hewinson, R.G., 2007. Assessment of cross-reactivity between *Mycobacterium bovis* and *M. kansasii* ESAT-6 and CFP-10 at the T-cell epitope level. *Clinical and Vaccine Immunology*, *14*(9), pp.1203-1209.

Warren, R.M., Gey van Pittius, N.C., Barnard, M., Hesseling, A., Engelke, E., De Kock, M., Gutierrez, M.C., Chege, G.K., Victor, T.C., Hoal, E.G. and Van Helden, P.D., 2006. Differentiation of Mycobacterium tuberculosis complex by PCR amplification of genomic regions of difference. *The International Journal of Tuberculosis and Lung Disease*, *10*(7), pp.818-822.

© University of Pretoria

Waters, W.R., Palmer, M.V., Buddle, B.M. and Vordermeier, H.M., 2012. Bovine tuberculosis vaccine research: historical perspectives and recent advances. *Vaccine*, *30*(16), pp.2611-2622.

Whipple, D.L., Bolin, C. A. and Miller, J.M., 1996. Distribution of lesions in cattle infected with *Mycobacterium bovis*. *Journal of Veterinary Diagnostic Investigation*, *8*(3), pp.351-354.

Woldemariam, T., Pal, M., Zewude, A., Mamo, G. and Gutama, G.A.K.P., 2021. A study on the prevalence of tuberculosis in cattle at selected abattoirs in Ethiopia. *Journal Research in Microbiology*, *2*(2), pp. 9-13.

Wood, P.R. and Jones, S.L., 2001. BOVIGAMTM: an in vitro cellular diagnostic test for bovine tuberculosis. *Tuberculosis*, *81*(1-2), pp.147-155.

World Health Organization, 2013. *Global tuberculosis report 2013*. World Health Organization.

World Health Organization, 2009. *Global tuberculosis control: epidemiology, strategy, financing: WHO report 2009*. World Health Organization.

World Health Organization, 2017. *Digital health for the End TB strategy: progress since 2015 and future perspectives: meeting report, 7-8 February 2017* (No. WHO/HTM/TB/2017.02). World Health Organization.

World Health Organisation, 2021. Global tuberculosis report. World Health Organisation

Zachariah, A., Pandiyan, J., Madhavilatha, G.K., Mundayoor, S., Chandramohan, B., Sajesh, P.K., Santhosh, S. and Mikota, S.K., 2017. Mycobacterium tuberculosis in wild Asian elephants, southern India. *Emerging infectious diseases*, *23*(3), p.504.

Zlot, A., Vines, J., Nystrom, L., Lane, L., Behm, H., Denny, J., Finnegan, M., Hostetler, T., Matthews, G., Storms, T. and DeBess, E., 2016. Diagnosis of tuberculosis in three zoo

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elephants and a human contact—Oregon, 2013. *Morbidity and Mortality Weekly Report*, *64*(52), pp.1398-1402.

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Chapter 3. Review of data on culture of *Mycobacterium* spp. from livestock and game in South Africa available at the TB laboratory of OVR, South Africa: A 10-year data analysis (2007-2016)

3.1. Abstract

Background: In South Africa, isolation of *Mycobacterium* species from veterinary samples and record-keeping are conducted at the Agricultural Research Council-Onderstepoort Veterinary Research (ARC-OVR) as a national laboratory. A retrospective study was conducted on the laboratory data at the Tuberculosis Laboratory between 2007 and 2016. Various tissue samples such as lungs, liver, spleen and different lymphnodes from livestock, wildlife, and environmental samples (i.e. water) were routinely tested using the culture-based method. *Mycobacterium* species verification was done by conducting a series of polymerase chain reaction (PCR) tests. The data sets were analyzed using Stata version 15 (StataCorp, College Station, TX, USA) for the prevalence of all *Mycobacterium* spp. in diagnostic samples submitted over this 10-year period. Factors, which may play a role in the transmission of mycobacteriosis, especially bovine tuberculosis (bTB) were identified.

Results: The current study revealed that the overall prevalence of *Mycobacterium* spp. was 18.47% (834/4516, 95% CI: 17.37 – 19.63) with individual species prevalence at 11.47%, 5.20%, 1.53%, 0.24%, and 0.02%, for *Mycobacterium bovis*, *Mycobacterium* Other Than Tuberculosis (MOTT) excluding *M. avium*, *Mycobacterium tuberculosis, Mycobacterium avium* and *Mycobacterium orygis,* respectively. The findings revealed that *M. bovis* was the most prevalent *Mycobacterium* species in both domestic animals at 62.26% and wildlife at 63.68%. The study revealed that mycobacteria were mostly isolated from samples collected

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from animals originating from Mpumalanga province [(n=514); 95% CI 4.12 – 7.67 (P<0.0001)] and most of the tissue samples were prone to infection. The presence of wildlife reservoirs and contact with these reservoirs, as well as environmental factors were among the factors identified that may have acted as potential causes of bTB transmission among the tested animal species.

Conclusion: The laboratory data provided an understanding of the occurrence of *Mycobacterium* spp. in both wildlife and livestock in South Africa and factors that are likely to influence the transmission of mycobacteriosis such as bTB. Improved data collection is required so that existing gaps highlighted by the information obtained from the records can be addressed by researchers.

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3.2. Introduction

The infection of wildlife and livestock with pathogens that causes bovine tuberculosis (bTB) has raised serious concerns globally. The disease is highly infectious with a wide host range consisting of wildlife, domestic and captive animals, and humans (Michel *et al*., 2010). The most commonly found members of the *Mycobacterium tuberculosis* complex (MTBC) species universally are the *Mycobacterium tuberculosis* and *Mycobacterium bovis. Mycobacterium tuberculosis* is responsible for TB in humans, while *Mycobacterium bovis* is the causative agent of the disease in animals (Pavlik *et al*., 2003; Smith *et al*., 2006). Transmission of the disease pathogens can occur from humans to animals and vice versa (Frietsche *et al*., 2004; Ameni *et al*., 2013). Other members of the MTBC include *Mycobacterium canetii, Mycobacterium pinnipedii, Mycobacterium caprae, Mycobacterium mungi, Mycobacterium orygis, Mycobacterium suricattae* and *Mycobacterium africanum* (Brosch *et al*., 2002; Warren *et al*., 2006; Rodriguez *et al*., 2011; Alexander *et al*., 2017). Transmission from animals to humans was reported to occur via inhalation of infectious droplets from an infectious source or via ingestion of unpasteurized dairy products or undercooked or raw meat from infected animals (Daborn *et al*., 1996; Kazwala *et al*., 2001)**.** The eradication of this disease is a major challenge, especially for developing countries due to insufficient control measures due to a lack of funds (Cosivi *et al*., 1998). In addition, the presence of an array of wildlife species that act as reservoirs for the disease and could re-infect the livestock also remains a huge threat (Michel, 2002).

In Africa, bTB remains one of the diseases that is underdiagnosed and under-researched, and as a result, there is insufficient knowledge of its prevalence (Dibaba & Daborn, 2019). In South Africa, bTB is a controlled disease in terms of the Animal Disease Act (Act 35 of 1984) and with the introduction of the Bovine Tuberculosis Eradication Scheme that was implemented in 1969, the prevalence of bTB was successfully reduced from 11.9% in 1971 to 0.4% in 1995

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(DAFF, 2013). Since then, the prevalence of bTB remained low in the cattle population in SA. In a recent study by Mareledwane *et al.* (2021), the prevalence of bTB in livestock at Gauteng Province abattoirs was determined using the interferon-gamma (IFN-γ) assay (Mareledwane *et al.*, 2021). The study reported that the estimated prevalence of cattle positive for bTB was 4.4%.

In developed countries such as New Zealand, the information regarding dominant risk factors that determine the infection and transmission of *M. bovis* is well documented (Baker *et al.*, 2006). Whereas, in Africa, there are many gaps in the database that contains information about the prevalence of bTB and the risk factors that promote the introduction and spread of the disease (Drewe *et al*., 2014). In South Africa, the TB laboratory at the ARC-OVR is the national laboratory approved by the Department of Agriculture, Land Reforms and Rural Development (DALRRD) and accredited by the South African National Accreditation System (SANAS) for the testing of bTB and the subsequently keeping records. The confirmation of the presence of infection is routinely done using the culture-based technique (which is considered a gold standard). For species verification PCR is used. The IFN-γ assay is used to identify infected animals.

Thus, the current study reviewed the laboratory data in South Africa, at the TB laboratory of the ARC-OVR, of slaughtered livestock and game (wild animals found dead in some instances) samples tested using culture-based technqiues.The study assessed the prevalence of all *Mycobacterium* spp. in diagnostic samples submitted at this laboratory over 10 years (2007-2016). The study identified associated factors, such as the presence of wildlife reservoirs, that play a role in the transmission of the disease.

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3.3. Materials and Methods

3.3.1. Study design

The study design was to obtain, arrange and analyze laboratory data of samples that were tested for mycobacteriosis mainly bTB in the TB laboratory at ARC-OVR. The data for livestock, wildlife, and environmental samples were collected from the laboratory records where all the information is recorded. The data set was extracted from laboratory records and arranged using Microsoft Excel version 2016. Dataset was arranged according to test results, provinces of origin of samples, years of sampling/testing, type of samples tested, and types of animal species where applicable.

3.3.2. Study area

South Africa is located in the continent of Africa and has an area of 1,219,602km^2 with a population estimated at 60.14 million in 2021 (Stats SA, 2011) . The country consists of nine provinces, Eastern Cape, Western Cape, Northern Cape, Limpopo, Mpumalanga, North-West, KwaZulu-Natal, Free State, and Gauteng. The Tuberculosis laboratory (ARC-OVR) is located in Gauteng province and samples that are submitted for testing do not only originate from the Gauteng provinces but the various provinces around the country and also from neighbouring African countries such as Botswana, Swaziland and Mozambique.

3.3.3. Sampling

Records from 2007-2016 used in the study were retrieved from the Tuberculosis Laboratory (ARC-OVR). The samples submitted were collected from- (a) animals that tested positive for TST and IFN-γ; (b) animals such as buffaloes within game farms/reserves with animals that had tested positive for IFN-γ and negative/positive for a skin test; (c) healthy-looking cattle displaying lesions during slaughter at abattoirs; (d) suspect animals detected during routine slaughter at abattoirs; and (e) dead wild animals found in game parks, in this case, tissues with

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pathological changes were collected and sent to the laboratory for culture testing (Hlokwe *et al*., 2014). Other types of samples that were submitted included environmental samples (i.e. soil and water). The samples were submitted together with a submission form containing information relating to the animal, owner, and location (Hlokwe *et al*., 2014).

3.3.4. Laboratory tests data

The routine mycobacterial culture and mycobacterium species verification by PCR were used for testing the samples that were submitted at the TB laboratory (ARC-OVR).

3.3.5. Statistical analysis

All data were entered and stored in Microsoft Excel version 2016. The retrospective data were analyzed using Stata version 15 (StataCorp, College Station, TX, USA). Proportions and percentages were generated with 95% confidence interval ($CI_{95\%}$). The risk of positivity for tuberculosis was compared using two by two tables. All statistically significant values were taken at p-value ≤ 0.05.

3.3.6. Ethical considerations

The study was approved by the Animal Ethics Committees for the Agricultural Research Council-Onderstepoort Veterinary Research (AEC 12.16) (Appendix 5.2) and the University of Pretoria (V104-17) (Appendix 5.3). Authorization to carry out the study at the abattoir was granted by the Department of Agriculture, Land Reforms and Rural Development (DALRRD) through section 20 approval (Appendix 5.4).

3.4. Results

3.4.1. Prevalence and risk analysis

In our study, we collected data available at the TB laboratory at the ARC-OVR from the years 2007-2016 (Figure 3.1). During this period, samples (n=4516) were tested using the microbial

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culture method, followed by species verification by a series of PCR tests. The month of April had the highest number of samples submitted through out the study period as compared to December which had the least number of samples submitted (Figure 3.1). Of the total number of samples tested, the total prevalence of all *Mycobacterium* spp. was found to be (834/4516) 18.47%; $p < 0.05$ (95% Confidence Interval: 17.37 – 19.63). *Mycobacterium* spp. that were isolated from the samples were: *M. bovis*, *M. tuberculosis*, *M. avium*, *M. orygis,* and the MOTT (Mycobacterium Other Than Tuberculosis), excluding *M. avium* (Table 3.1). From samples that tested positive for Mycobacterium species (n=834), *M. bovis* was the most prevalent (62.11%) followed by the MOTT (excluding *M. avium*) at 28.18% and *M. orygis* was the least prevalent *Mycobacterium* spp. (Table 3.2). Of the MTBC species isolated, *M. bovis* was the more prevalent species detected in both domestic animals at 62.26% and wildlife at 63.68%, followed by *M. tuberculosis* detected in 4.72% of the domestic animals, and 9.8% of the land wildlife species. *M. orygis* was detected in just one animal. Avian tuberculosis is caused by *M. avium*, hence all mycobacterium spp. isolated from birds were identified as such as shown in Table 3.3.

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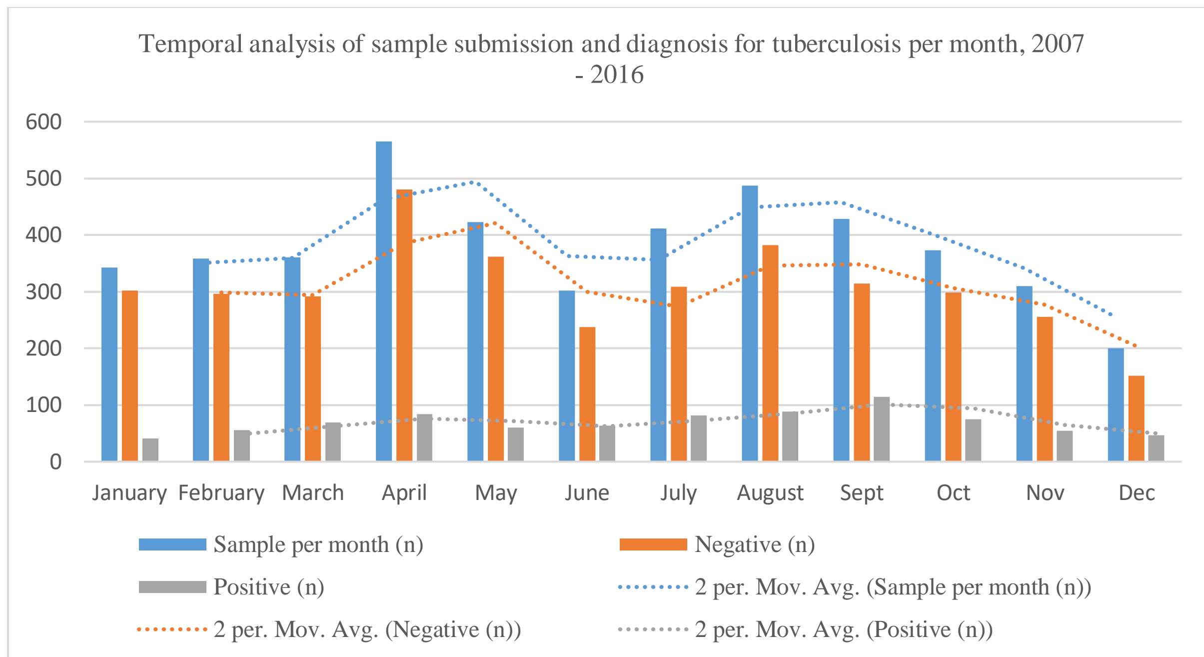

Figure 3.1: Temporal analysis of sample submission and diagnosis of mycobacteriosis including-bovine tuberculosis per month, 2007-2016.

Table 3.1: Prevalence of *Mycobacterium* spp. in the animals tested during the period 2007 to 2016 on positive samples.

Species of *Mycobacterium*	**Total number of animals tested**	**Prevalence (%)[a]**
M. tuberculosis	69	1.53
M. bovis	518	11.47
M. avium	11	0.24
M. orygis	1	0.02
MOTT	235	5.20
p-value		<0.05

(n=4516): A total number of samples tested with a total prevalence of all *Mycobacterium* spp. being 18.47% (834/4516) (95% Confidence Interval: 17.37 – 19.63).

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Table 3.2: Identification of *Mycobacterium* spp. in positive samples

Identification of *Mycobacterium* spp. isolated	**Total number positives**	**Percentage (%)[a] of positive samples**	**$CI_{95\%}$ (%)**
M. tuberculosis	69	8.27	6.58 – 10.35
M. bovis	518	62.11	58.77 – 65.34
M. avium	11	1.32	0.71 – 2.38
M. orygis	1	0.12	0.01 – 0.75
MOTT	235	28.18	25.23 – 31.33

[a]Of a total of 834 positive samples

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Table 3.3: Classification of positive samples by groupings, *i.e*., domestic animals, wildlife other than birds and water wildlife, wild birds, animals in water, and environmental samples.

Classification of positive samples by groupings	**Number positive**	**Percentage (%)**	**Number positive**	**Comments (if any)**
Variable (n)	***M. bovis* (%)**	***M. tuberculosis* (%)**	**Others (%)**	**Comments**
Domestic animals[1] (212/1790)	132 (62.26)	10 (4.72)	70 (33.02)	Others are inclusive of MOTT (64), *M. avium* (5), and unclassified (1)
Wildlife[2] (other than birds and in water) (603/2723)	384 (63.68)	59 (9.78)	160 (26.53)	Others are inclusive of MOTT (158) and *M. orygis* (1) and unclassified (1)
Wild avian (birds)[3] (6/17)	0 (0.0)	0 (0.0)	6 (100.00)	All positive isolates were *M. avium*
Animals in water[4] (6/12)	0 (0.0)	0 (0.0)	6 (100.00)	All positive isolates were MOTT
Environmental samples[5] (7/20)	0 (0.0)	0 (0.0)	7 (100.00)	All positive isolates were MOTT

[1]Domestic animals include bovine, samples from bovine, caprine, canine, rabbits, Guinea pigs, equine and porcine.
[2]Wildlife include the following: lions, Mandarin baboons, Patas monkeys, monkeys, impalas, baboons, Waterboks, Buffalos, Chacma baboons, leopards, Axis deers, Black wildebeests, deer, Kudus, Red hartbees, Vervet monkeys, White rhinos, hyenas, African buffalos, Bushbucks, Blue wildebeests, Mongooses, Nyalas, African civets, elephants, Cheetahs, giraffe, African wild dogs, Black rhino, Klipspringer, Honey badgers, spotted hyena, banded mongooses, warthog, unidentified primate, genets, tortoises, chimpanzees, wild guinea pigs, meerkats, springboks, lesser kudus, gorilla, oryx, African wild cats, servals, tsessebe, bucks, black-banded jackals, Asian water monitors, Nubian ibexes, rock hyena, elands, Blesboks, duikers, beberage, These were further sub-divided into carnivores, herbivores and omnivores.
[3]Wild avian include parrots, crowned plover, various avian species, crested barbet, amazon parrot, Hadeda ibis, crowned lapo, little swift, cranes, African grey parrots, and croconed crate.
[4] Animals in water include Cape fur seal, fishes (Cruppy, exotic, and Oscar), and frog.
[5]*Environmental samples are soil, slurry, water, bees, feed, and hay.*

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3.4.2. Risk analysis of *Mycobacterium* spp in nine South African provinces

A comparison of the risk of detection/confirmation of *Mycobacterium* spp from the nine different provinces showed that Limpopo, Gauteng, and Mpumalanga provinces had the highest risk of detection of 8-fold, 7-fold, and 5-fold respectively, meaning they are more likely to produce positive samples as compared to Eastern Cape, which had the lowest risk of detection. The risk of detection of *Mycobacterium* species was however, statistically significant in samples originating from eight of the nine provinces Free State, Gauteng, KwaZulu Natal, Limpopo, Mpumalanga, North West, Western Cape and Northern Cape Provinces when compared with Eastern Cape Province (Table 3.4).

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Table 3.4: Comparison of the risk of detection/confirmation of *Mycobacterium* spp. in South Africa.

classification by provinces (n)[a]	Positive	Negative	Conditional maximum likelihood estimate of Odds Ratio (CMLE OR)	Confidence Interval$_{95\%}$ (*p-value*)
EC[b] (n=862)	48	814	1.00	NA
FS[c] (n=113)	20	93	3.64	2.04 – 6.36 (<0.0001)
GP[d] (n=244)	70	174	6.81	4.56 – 10.22 (<0.0001)
KZN[e] (n=329)	35	294	2.12	1.27 – 3.18 (0.003)
LP[f] (n=198)	65	133	8.26	5.46 – 12.58 (<0.0001)
MP[g] (n=2061)	514	1547	5.64	4.12 – 7.67 (<0.0001)
NW[h] (n=79)	10	69	2.46	1.19 – 4.95 (0.03)
WC[i] (n=100)	14	86	2.76	1.42 – 5.13 (0.004)
NC[j](n=464)	51	413	2.09	1.39 – 3.17 (0.005)

[b]EC: Eastern Cape, [c]FS: Free State, [d]GP: Gauteng Province, [e]KZN: KwaZulu Natal, [f]LP: Limpopo, [g]MP: Mpumalanga, [h]NW: North-west, [i]: Western Cape, [j]NC: Northern Cape

Total number of samples tested from the nine provinces (n=4450).

Analysis showed that more samples were submitted to the laboratory for analysis between the periods 2007-2009 as compared to years 2010-2016. According to DALRRD, the highest

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number of bTB outbreaks was reported in 2003 and 2009, corresponding with highest number of samples submitted for testing at this particular period (Table 3.5a; Figure 3.1).

Table 3.5a: Risk classification of positive and negative samples by year

Risk classification by year	**Positive**	**Negative**	**CMLE OR**	**Confidence Interval$_{95\%}$ (*p-value*)**
2007 (n=725)	107	618	1.26	0.87 – 1.82 (0.22)
2008 (n=754)	186	568	2.37	1.69 – 3.37 (<0.0001)
2009 (n=766)	147	619	1.72	1.22 – 2.46 (0.002)
2010 (n=304)	80	304	1.91	1.29 – 2.83 (0.001)
2011 (n=264)	64	264	1.76	1.17 – 2.65 (<0.01)
2012 (n=287)	29	287	0.73	0.45 – 1.19 (0.21)
2013 (n=184)	51	184	2.01	1.30 – 3.10 (<0.002)
2014 (n=150)	33	150	1.59	0.98 – 2.58 (0.06)
2015 (n=348)	48	348	1.00	NA
2016 (n=339)	89	339	1.90	1.30 – 2.80 (<0.001)

(n=4121): Total number of the types of samples tested

On the temporal analysis of the sample submission and diagnosis for tuberculosis per month for the period between 2007 and 2016, the trend for the positive samples is almost the same for all the months as they are below 100, except for the September months (Table 3.5b).

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Table 3.5b: Risk classification of positive and negative samples by month

Risk classification by month	Positive	Negative	CMLE OR	Confidence Interval$_{95\%}$ (*p-value*)
January	41	302	0.78	0.52 – 1.15 (0.21)
February	56	296	1.08	0.75 – 1.56 (0.68)
March	69	292	1.35	0.95 – 1.92 (0.09)
April	84	480	1.00	NA
May	60	362	0.95	0.66 – 1.35 (0.77)
June	64	238	1.54	1.07 – 2.20 (0.02)
July	82	309	1.52	1.08 – 2.12 (0.02)
August	88	382	1.32	0.95 – 1.83 (0.10)
September	114	314	2.07	1.51 – 2.85 (<0.0001)
October	75	298	1.44	1.02 – 2.03 (0.04)
November	54	256	1.21	0.83 – 1.75 (0.33)
December	47	152	1.77	1.18 – 2.63 (<0.01)

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Temporal analysis shows sample submission and diagnosis for tuberculosis per year (2007-2016) and the trend demonstrated that there was a high number of samples collected between 2007-2009 as compared to 2010-2016. In addition, the trend shows that between 2007-2009, there were a lot of samples from wildlife that were submitted for testing as compared to the other years as shown in Figure 3.2 below. Also, the data revealed that the risk of *Mycobacterium* spp. detection was higher in water wildlife species, environmental samples, wild avian and land wildlife, with the risk of detection being 7-fold, 6-fold, 4-fold, and 2-fold, respectively as compared to the risk of detection in domestic animals (1 fold risk of detection) (Table 3.6).

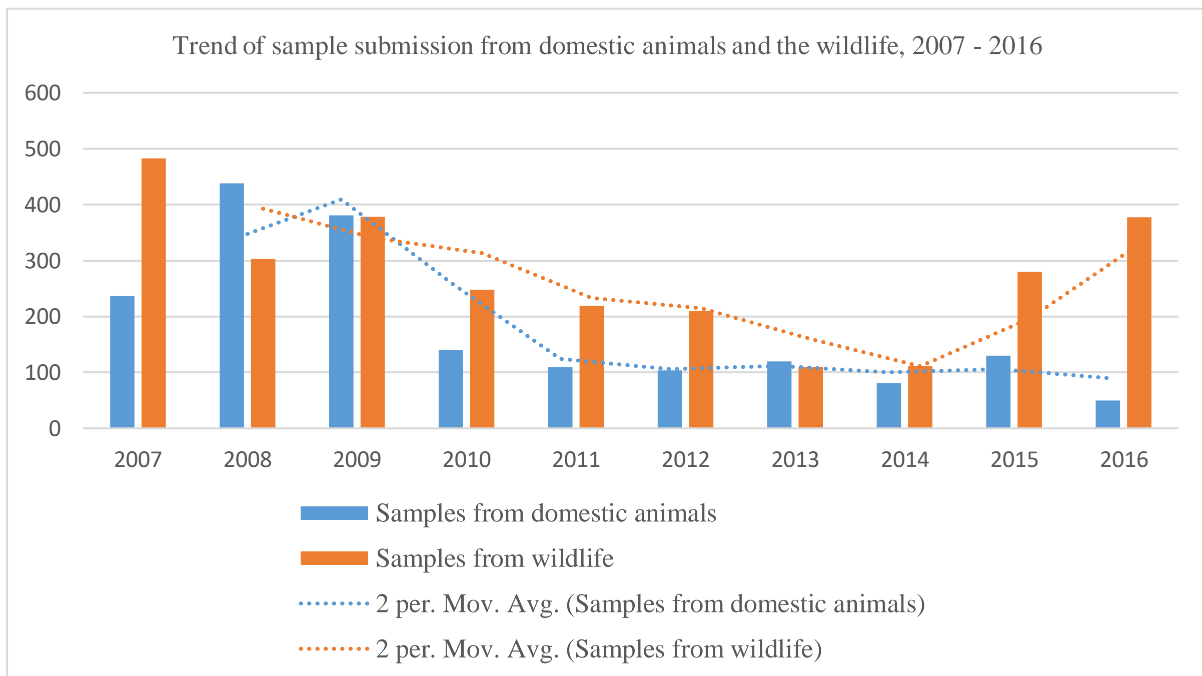

Figure 3.2: Trends of sample submission from domestic animals and the wildlife in the Tuberculosis laboratory (2007-2016).

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Table 3.6: Risk classification of positive and negative samples by groupings and feeding habits.

Risk classification by groupings	Positive	Negative	CMLE OR	Confidence Interval$_{95\%}$ (*p-value*)
Domestic animals (n=1783)	212	1571	1.00	NA
Wildlife (other than birds and in water) (n=2688)	603	2085	2.14	1.81 – 2.54 (<0.0001)
Wild avian (n=17)	6	11	4.04	1.37 – 11.00 (0.01)
Wildlife in water (n=12)	6	6	7.40	2.29 – 24.45 (0.002)
Environmental samples (n=15)	7	8	6.47	2.21 – 18.57 (0.001)

(n=4515): Total number of samples tested from all sources

3.4.3. Risk classification of positive and negative samples in wildlife, wild environment, and domestic animals

Amongst the samples collected from the wildlife, the risk of detection was most high in wildlife in the water (risk factor almost 4 times) while mongooses had the least risk, with odds of 0.26. Additionally, data showed that the risk of infection was statistically significant in wild predators ($p<0.01$) and herbivores and omnivores ($P=0.001$), see Table 3.7.

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Table 3.7: Risk classification of positive and negative samples in wildlife and wild environment

Risk in the wild [total number of samples]	Positive	Negative	CMLE OR	Confidence Interval$_{95\%}$ (*p-value*)
Wild carnivores (predators) [n=882]	226	656	1.36	1.08 – 1.72 (<0.01)
African wild buffaloes (herbivores) [n=743]	150	593	1.00	NA
Other wild ruminants (herbivores)/omnivores [n=731]	200	531	1.49	1.17 – 1.90 (0.001)
Mongooses [n=310]	19	291	0.26	0.15 – 0.42 (<0.0001)
Environmental samples collected from the wild environment [n=22]	8	14	2.26	0.88 – 5.46 (0.08)
Wild avian [n=17]	6	11	2.15	0.73 – 5.90 (0.16)
Other environmental samples [n=15]	7	8	3.45	1.17 – 9.96 (0.03)
Wildlife in water [n=12]	6	6	3.94	1.19 – 13.10 (0.03)

[n=2732]: Total number of types of samples tested

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Overall, 1783 domestic animal samples, with over 94% collected from cattle (n=1684), were tested and results analysed. Of all the cattle samples analysed, 11% were positive for *mycobacterium* species and 26% (n=26) of the other domestic animals (n=99) also tested positive for *mycobacterium* species. Risk analysis of *mycobacterium* spp. detection in domestic animals indicates that the risk of detection is approximately 7times higher in other domestic animals combined than domestic bovine (cattle) as shown in table as shown in Table 3.8 below.

Table 3.8: Risk classification of positive and negative samples in domestic animals

Risk in domestic animals	**Positive**	**Negative**	**CMLE OR**	**Confidence Interval$_{95\%}$ (*p-value*)**
Domestic bovine (n=1684)	186	1498	1.00	NA
Other domestic animals combined (n=99)	26	73	6.79	4.75 – 10.13 (<0.0001)

(n=1783): Total number of samples tested.

3.4.4. Risk analysis of *Mycobacterium* spp. detection based on organs and body systems

Out of 3015 samples from a variety of lymph nodes, tonsils, and lymphatic tissues analysed, 531 samples tested positive for *mycobacterium* species and 2484 negative. Other related sample types (whole carcasses, muscles, unidentified tissues, tissue homogenates; n=159) were also analysed and 41 were found to be positive for *mycobacterium* species. The risk of *mycobacterium* spp. infection was found to be statistically significant ($p<0.0001$) in all these sample matrices. A *mycobacterium* species was more likely to be detected ($p<0.0001$) in respiratory tract samples (i.e. Lung, bronchial wash, bronchial effusion, lung milt, lung granuloma, pharynx, trachea, tracheal fluid, tracheal flushing, thoracic effusion, thorax, heart). Isolation was made from 146 of these type samples from a total of 530 that was analysed (Table 3.9).

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Table 3.9: Risk analysis of positive and negative samples based on organs and body systems.

Risk classification based on organs and systems (Total number of samples)	Positive	Negative	CMLE OR	Confidence Interval$_{95\%}$ (*p-value*)
Lymph nodes, tonsils, and lymphatics (n=3015)	531	2484	5.04	2.90 – 9.47 (<0.0001)
Milk (15)	0	15	-	NA
Skin, feet, and mammary swab (n=6)	1	5	4.67	0.18 – 37.20 (0.26)
Abdominal, visceral and the digestive system (n=270)	43	227	4.46	2.34 – 8.98 (<0.0001)
Lung, bronchial wash, bronchial effusion, lung milt, lung granuloma, pharynx, trachea, tracheal fluid, tracheal flushing, thoracic effusion, thorax, heart (n=530)	146	384	8.95	5.01 – 17.16 (<0.0001)
Abscess from liver, lung, and other tissues (n=22)	3	19	3.70	0.78 – 13.57 (0.09)
Aspirates (bloody, mucoid, testicular, tibial), biopsy, fluids, exudate, oedema (n=14)	4	10	9.27	2.24 – 33.77 (0.04)
Brain, spinal cord, and head (n=28)	7	21	7.76	2.62 – 21.93 (0.0005)
Whole carcass, muscle, tissue, tissue homogenate (n=159)	41	118	8.15	4.21 – 16.66 (<0.0001)
Cotyledons, placenta, uterus, kidney (n=16)	5	11	10.53	2.89 – 35.33 (<0.001)
Faeces, urine, hay, water, soil (n=40)	15	25	13.93	5.86 – 33.81 (<0.0001)
Hygroma, swabs, impression smears, pleura, slides (n=93)	20	73	6.42	3.01 – 14.13 (<0.0001)
Saliva, tongue, salivary glands and sputum (n=295)	12	283	1.00	NA
Elbow, knee joints, inguinal, others (unclassified) (n=13)	6	7	19.64	5.45 – 70.31 (<0.0001)

(n=4516): Total number of samples tested

3.5. Discussion

In this study, we retrieved and analysed data from the ARC-OVR (Tuberculosis Laboratory) to determine the occurrence of *Mycobacterium* species causing mycobacterisis including bTB in South African domestic and wildlife animals. This study focused mainly on determining the overall presence of *Mycobacterium* spp. and elements that may be associated with the transmission of bTB. Abattoir and laboratory data are mainly used as sources of surveillance systems (Arnot & Michel, 2020). In an abattoir-based retrospective study in Algeria (2009-2018), Ayad *et al*. (2020), determined the occurrence of tuberculosis and the data obtained provided an insight into the magnitude of bTB in the Bejaia Province in Algeria. However,

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another retrospective study by Sa'idu *et al.* (2017) noted that data available on abattoir records may underestimate the true prevalence of the disease. The authors suggested that the findings may be of a lack of proper surveillance and reporting. In South Africa, bTB has been documented in all nine provinces and sporadic disease reports indicating bTB cases involving different wildlife were documented but the prevalence in the nine provinces was not determined (Hlokwe *et al.*, 2014; Dibaba & Kriek, 2019). Therefore, information on bTB is still limited.

The current study revealed that the total prevalence of *Mycobacterium* spp. in samples submitted at the TB laboratory during the study period 2007-2016 was 18.47% (834/4516) with individual *Mycobacterium* strain prevalence at 11.47%, 5.20%, 1.53%, 0.02%, and 0.02%, for *M. bovis*, MOTT (excluding *M. avium)*, *M. tuberculosis*, *M. avium*, *M. orygis* respectively. . The isolation of *M. bovis* 62.26% (in domestic animals and 63.68% wildlife) being the most prevalent pathogen, from the samples, shows that both domestic animals and wildlife are the source of infection in South Africa, (Renwick, 2007). In SA, bTB is controlled through the test and slaughter approach, and this programme has since reduced the prevalence of the disease in cattle to <1% (Michel *et al.*, 2008). Sporadic outbreaks still occur as reported by Hlokwe and co-workers (Hlokwe *et al.*, 2014). In other African countries such as Kenya and Uganda, cattle are reported to be the source of bTB disease transmission to wildlife animals. Gathogo *et al.* (2012) documented for the first time in Kenya, that the presence of bTB in slaughtered cattle and the prevalence of *M. bovis* was estimated at 2.05% (95% Confidence Interval: 1.24 - 3.18). Another study by Ameni *et al.* (2010), reported the isolation of *M. bovis* strains from tuberculous lesions in cattle in Ethiopia (Ameni *et al.*, 2010), indicating that the disease is endemic in some parts of Africa. The overall prevalence of *Mycobacterium* spp. in this study was 18.47% (834/4516), however; other published studies like in Ningo, a region in Ghana, the prevalence was higher at 50% and in Tanzania, prevalence among different districts ranged between 0.19%-14% (Daborn & Grange, 1993; Bonsu *et al.*, 2000).

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The present study revealed that *M. bovis* was the most prevalent *Mycobacterium* species in wildlife, with a prevalence of 63.68%. It is important to note that 56.71% of all positive wildlife were buffaloes, however, based on the current risk of infection conducted, buffaloes, as well as other wild ruminants, wild carnivores were equally exposed to infection by *M. bovis*. This can be due to intense interaction, sharing of space in the ecosystem, and the food chain which links them together as observed in other studies (Ayele *et al*., 2004). In SA, buffaloes are considered the wildlife maintenance hosts for bTB, meaning that they can also harbour the infection for many years before any clinical signs of the disease can be observed (Getahun, 2017). Hlokwe *et al*. (2011) isolated three *M. bovis* strains from individual buffaloes originating from the HiP game reserve, KNP as well as in private game reserves (Hlokwe *et al*., 2014 and 2016). Spill-over from buffaloes to other wildlife animals was also reported. Phepa *et al*. (2016) reported that eradication of bTB is impossible as long as buffalo and cattle are in contact with one another at domestic animals-wildlife interfaces. Therefore, our findings further supports previous suggestions that *M. bovis* is harboured by both the wildlife (buffaloes) and livestock (cattle). In South Africa, evidence of spill back from buffalo back to cattle has been reported by Musoke *et al*. (2015). This is one of the factors that drive the persistence of the disease in the wildlife-livestock interface posing a danger to spill-back and spill-over of the disease between the species (Musoke *et al*., 2015).

Based on the risk classification of samples originating from different provinces, the current study revealed that samples from Limpopo, Gauteng, and Mpumalanga provinces had a high risk of *Mycobacterium* species detection of 8-fold, 7-fold, and 5-fold respectively as compared to the Eastern Cape, which had a risk of detection 1-fold. This may suggest that animals from provinces such as Limpopo, Gauteng, and Mpumalanga are more likely to be infected with mycobacteriosis since they have the highest risk of *Mycobacterium* species detection. The finding that Gauteng was the province that had the second-highest likehood of detection may

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be due to the fact the Tuberculosis Laboratory ARC-OVR is based in Pretoria and is within reach of most facilities making it convenient for the transportation of suspect samples that needed bTB confirmed, as suggested in other diseases prevalence investigations (Kolo *et al*., 2020). The majority of samples from Gauteng province originated from the Johannesburg and Pretoria National zoological gardens and *M. tuberculosis* rather than *M. bovis* was isolated. Kolo *et al*. (2020) also noted the same scenario whereby Gauteng Province had the highest seropositive samples for brucellosis as compared to other provinces. Limpopo and Mpumalanga were the other two provinces that had a high had a high risk of detection of positive samples. This was attributed to the fact that South Africa's biggest game reserve, the Kruger National Park (KNP), is located in parts of both provinces, i.e., Mpumalanga and Limpopo, and has adjacent private game reserves with rural cattle farms bordering them. Bovine tuberculosis (bTB) is endemic in the KNP, with the prevalence higher in the southern part of the reserve (Hlokwe *et al*., 2014). Previous findings have indicated that there are negative implications in livestock-wildlife interactions, especially in rural communities majoring in cattle farming bordering bTB endemic buffaloes in game reserves (Musoke *et al*. 2015). In South Africa, a study by Sichewo *et al*. (2020) investigated the wildlife-livestock interface between communal farmland and surrounding game parks in KwaZulu-Natal province of S.A. The findings revealed five *M. bovis* VNTR genotypes shared between buffalo and cattle, demonstrating *M. bovis* transmission between species.

The risk classification of positive samples based on groupings by feeding habits showed that the risk of *Mycobacterium* spp. detection in samples collected from wild carnivores and wild ruminants (herbivores other than buffaloes as well as omnivores) was statistically significant, $p<0.01$ and $p=0.001$ respectively. Samples collected from wildlife in the water had the highest risk of *Mycobacterium* spp. detection. This can be due to aquatic predators feeding on weaker/sickly infected dead prey in water leading to the shedding of the pathogen (De Garine-

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Wichatitsky *et al*., 2013). An example can be a crocodile preying on an infected buffalo when they congregate in drinking areas (De Garine-Wichatitsky *et al*., 2013). The consumption of infected material such as lesions and organs leads to the transmission of the bacteria and becomes one of the reasons that wildlife in water has a high risk of detection (De Garine-Wichatitsky *et al*., 2013). Thus, predator-prey interactions are one of the drivers in the transmission of bTB hence the high risk of detection in samples that were collected from wildlife in water (De Garine-Wichatitsky *et al*., 2013). The other factor which was observed to have a high risk of detection was the environmental samples (6-fold risk of detection). This can be due to infected animals shedding *Mycobacteria* into the environment, and transmission may occur through faeces or urine of infected animals. Contaminated material is then consumed by other animals resulting in transmission of *Mycobacterium* spp. (Renwick *et al*., 2007).

Although most *Mycobacterium* spp. were isolated more in cattle than any other domestic animal, the results show that the risk of detection is higher in other domestic species combined as compared to cattle. This phenomenon suggests that research should be conducted for other domestic animals especially those that are susceptible to the disease and not only be confined to domestic bovines alone. Ramdas *et al*. (2015), described the isolation of *M. bovis* from two indoor cats. In another study, Roca *et al*. (2017) isolated *M. bovis* in a dog in Brazil. The studies concluded that although there is low transmission between humans and pets, dogs and cats living in close contact with *M. bovis* infected cattle should be screened (Roca *et al*., 2017). Greene (2006) suggested that domestic species other than bovine species play a role in the maintenance of *M. bovis* in a farm setting. The samples in the data were from routine sampling which normally focused on the routine surveillance of domestic bovines neglecting other domestic species. It is thus important to create long term surveillance programs that are inclusive of other domestic species and conduct routine sampling on other domestic animals especially those living in close proximity with resevoirs or hosts of bTB. Also, there is a need

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for expansion of testing requirements and develop diagnostic tests for other species that are susceptible to bTB. In Africa, livestock farming communities practise mixed livestock systems, and this means close contact between domestic animals such as sheep, goats, and domestic bovines. Therefore, when the cattle become infected it spills over to other domestic animals (Muwonge *et al*., 2012). It is recommended that routine surveillance and sampling should be incorporated into national control programs.

Observation from a risk analysis of positive samples based on organs and systems suggests that tuberculosis affects all organs. This is in line with the literature which states that tuberculous lesions are systematic and can be detected in various tissue organs (Cosivi *et al*., 1995). Infected animals contaminate the environment by discharging contaminated faecal and nasal material into running water (Humblet *et al*., 2009). Apart from samples obtained from lymph nodes, samples of skin, feet, and whole carcass as shown in Table 3.9, had a high risk of detection compared to saliva which was less likely to be positive. The likelihood of detecting Mycobacterial spp. from cotyledons, placenta, uterus and kidneys was high in this study, and could be an indication that the pathogen can cross the uterus and infect the foetus in pregnant females. This finding is in line with a study by Karamian *et al.* (2020), in their finding they established the presence of *M. bovis* in the placenta and foetal fluid. They concluded that *M. bovis* could have been ingested from the amniotic fluid.

In the current study, more samples were submitted for analysis in the Tuberculosis laboratory at OVR-OVR between the periods 2007-2009 as compared to the period 2010-2016. According to DALRRD, the highest numbers of bTB outbreaks were reported in 2003 and 2009, hence this is consistent with the high number of laboratory submissions in 2009 (Arnot & Michel, 2020). It should however, be noted that the temporal analysis of sample submission for bTB testing is not necessarily a good indicator of the risk of bTB occurrence in SA. Sampling in this study is biased because samples submitted for testing may be because animals were sick,

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hence culled and samples submitted for testing. Therefore, the actual prevalence of the disease may be underestimated, and thus, the true prevalence higher than reported. However, laboratory data may assist in the identification of trends responsible for the transmission of bTB.

Several factors significantly impacted the transmission of bTB and it is, therefore, important to understand these risk factors and their roles in disease transmission. Information from the laboratory data, not only revealed the occurrence of *Mycobacterium* spp. but also identified several factors associated with the transmission of bTB.

3.6. Conclusion

The findings of the study have provided baseline data for factors that may be responsible for the transmission and development of disease (bTB) in both wildlife and livestock in Gauteng province and the country at large. Limitations of the study included the location and coverage of the laboratory, which in most instances, is biased towards city populations (Cosivi, 1998). Improved data collection is required so that researchers can target several aspects highlighted by the information obtained from the records. Furthermore, the data may be biased because the sampling method used which may have only focused on animals that showed clinical signs (although not always the case with bTB) or on animals that were tested for the disease before translocation. In general, the identified factors are consistent with the transmissions and such knowledge could aid to minimise the risk of bTB transmission.

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3.7 References

Alexander, K.A., Laver, P.N., Williams, M.C., Sanderson, C.E., Kanipe, C. and Palmer, M.V., 2018. Pathology of the emerging Mycobacterium tuberculosis complex pathogen, Mycobacterium mungi, in the banded mongoose (Mungos mungo). *Veterinary pathology*, *55*(2), pp.303-309.

Ameni, G., Desta, F. and Firdessa, R., 2010. Molecular typing of Mycobacterium bovis isolated from tuberculosis lesions of cattle in north eastern Ethiopia. *Veterinary Record*, *167*(4), pp.138-141.

Ameni, G., Tadesse, K., Hailu, E., Deresse, Y., Medhin, G., Aseffa, A., Hewinson, G., Vordermeier, M. and Berg, S., 2013. Transmission of Mycobacterium tuberculosis between farmers and cattle in central Ethiopia. *PLoS One*, *8*(10), p.e76891.

Arnot, L.F. and Michel, A., 2020. Challenges for controlling bovine tuberculosis in South Africa. *Onderstepoort Journal of Veterinary Research*, *87*(1), pp.1-8.

Ayad, A., Bensid, A., Benabdelhak, A. C., Ait-Yahia, F., Dergal, N. B. (2020). First report on tuberculosis based on slaughterhouse data in Bejaia Province, Algeria: A retrospective 10-year survey. *Kocatepe Veterinary Journal*, 13(2), 118-124.

Baker, M.G., Lopez, L.D., Cannon, M.C., De Lisle, G.W. and Collins, D.M., 2006. Continuing Mycobacterium bovis transmission from animals to humans in New Zealand. *Epidemiology & Infection*, *134*(5), pp.1068-1073.

Bonsu, O.A., Laing, E. and Akanmori, B.D., 2000. Prevalence of tuberculosis in cattle in the Dangme-West district of Ghana, public health implications. *Acta Tropica*, *76*(1), pp.9-14.

Brosch, R., Gordon, S.V., Marmiesse, M., Brodin, P., Buchrieser, C., Eiglmeier, K., Garnier, T., Gutierrez, C., Hewinson, G., Kremer, K. and Parsons, L.M., 2002. A new evolutionary scenario for the Mycobacterium tuberculosis complex. *Proceedings of the National Academy of Sciences*, *99*(6), pp.3684-3689.

© University of Pretoria

Cosivi, O., Meslin, F.X., Daborn, C.J. and Grange, J.M., 1995. Epidemiology of Mycobacterium bovis infection in animals and humans, with particular reference to Africa. *Revue scientifique et technique (International Office of Epizootics)*, *14*(3), pp.733-746.

Cosivi, O., Grange, J.M., Daborn, C.J., Raviglione, M.C., Fujikura, T., Cousins, D., Robinson, R.A., Huchzermeyer, H.F., de Kantor, I. and Meslin, F.X., 1998. Zoonotic tuberculosis due to *Mycobacterium bovis* in developing countries. *Emerging infectious diseases*, *4*(1), p.59.

Daborn, C.J., Grange, J.M. and Kazwala, R.R., 1996. The bovine tuberculosis cycle—an African perspective. *Journal of Applied Bacteriology*, *81*, pp.27S-32S.

Daborn, C.J. and Grange, J.M., 1993. HIV/AIDS and its implications for the control of animal tuberculosis. *British Veterinary Journal*, *149*(5), pp.405-417.

De Garine-Wichatitsky, M., Caron, A., Kock, R., Tschopp, R., Munyeme, M., Hofmeyr, M. and Michel, A., 2013. A review of bovine tuberculosis at the wildlife–livestock–human interface in sub-Saharan Africa. *Epidemiology & Infection*, *141*(7), pp.1342-1356.

Department of Agriculture, Forestry and Fisheries (DAFF), Republic of South Africa. (2013). Interim bovine tuberculosis scheme manual. (6 December 2013). http://ww.nda.agric.agric.za/vetweb/pamphlets&Information/Policy/TB

Dibaba, A.B. and Daborn, C.J., 2019. Epidemiology of bovine tuberculosis in Africa. In *Tuberculosis in animals: An African perspective* (pp. 89-126). Springer.

Drewe, J.A., Pfeiffer, D.U. and Kaneene, J.B., 2014. Epidemiology of Mycobacterium bovis. *Zoonotic tuberculosis: Mycobacterium bovis and other pathogenic mycobacteria*, pp.63-77. Wiley-Blackwell.

Fritsche, A., Engel, R., Buhl, D. and Zellweger, J.P., 2004. *Mycobacterium bovis* tuberculosis: from animal to man and back. *The International Journal of Tuberculosis and Lung Disease*, *8*(7), pp.903-904.

© University of Pretoria

Gathogo, S.M., Kuria, J.K. and Ombui, J.N., 2012. Prevalence of bovine tuberculosis in slaughter cattle in Kenya: a postmortem, microbiological and DNA molecular study. *Tropical Animal Health and Production*, *44*(7), pp.1739-1744.

Getahun, A., (2017). Molecular characterization of Mycobacterium bovis and its significance: Role for control of zoonotic tuberculosis in Africa. *Journal of Medical Diagnostic Methods,* 6, 1-10.

Greene, C.E., 2006. *Infectious diseases of the dog and cat* (No. Ed. 3). WB Saunders\Elsevier Science.

Hlokwe, T.M., Jenkins, A.O., Venter, E.H., Michel, A.L., Streicher, E.M., Cooper, D. and Godfroid, J., 2011. Molecular characterisation of *Mycobacterium bovis* isolated from African buffaloes (Syncerus caffer) in Hluhluwe-iMfolozi Park in KwaZulu-Natal, South Africa. *Onderstepoort Journal of Veterinary Research*, *78*(1), pp.1-6.

Hlokwe, T.M., Van Helden, P. and Michel, A.L., 2014. Evidence of increasing intra- and inter-species transmission of *Mycobacterium bovis* in South Africa: are we losing the battle? *Preventive Veterinary Medicine*, *115*(1-2), pp.10-17.

Hlokwe, T.M., De Klerk-Lorist, L.M. and Michel, A.L., 2016. Wildlife on the move: a hidden tuberculosis threat to conservation areas and game farms through introduction of untested animals. *Journal of Wildlife Diseases*, *52*(4), pp.837-843.

Humblet, M.F., Boschiroli, M.L. and Saegerman, C., 2009. Classification of worldwide bovine tuberculosis risk factors in cattle: a stratified approach. *Veterinary Research*, *40*(5), pp.1-24.

Kazwala, R.R., Daborn, C.J., Sharp, J.M., Kambarage, D.M., Jiwa, S.F.H. and Mbembati, N.A., 2001. Isolation of Mycobacterium bovis from human cases of cervical adenitis in Tanzania: a cause for concern? *The International Journal of Tuberculosis and Lung Disease*, *5*(1), pp.87-91.

Karamian, M., Soleimanzadeh, A., Tukmechi, A. and Batavani, R. A., 2020. PCR investigation of the vertical transmission of *Mycobacterium bovis* in Montbeliarde cattle in Gonbad, Northeast of Iran. *Bulgarian Journal of Veterinary Medicine*, (online first).

© University of Pretoria

Kolo, F.B., Adesiyun, A.A., Fasina, F.O., Potts, A., Dogonyaro, B.B., Katsande, C.T. and Van Heerden, H., 2021. A retrospective study (2007–2015) on brucellosis seropositivity in livestock in South Africa. *Veterinary Medicine and Science*, *7*(2), pp.348-356.

Mareledwane, V.E., Adesiyun, A.A., Thompson, P.N. and Hlokwe, T.M., 2021. Application of the gamma-interferon assay to determine the prevalence of bovine tuberculosis in slaughter livestock at abattoirs in Gauteng, South Africa. *Veterinary Medicine and Science*, *00,* pp. 1-8.

Michel, A.L., Müller, B. and Van Helden, P.D., 2010. *Mycobacterium bovis* at the animal–human interface: A problem, or not? *Veterinary Microbiology*, *140*(3-4), pp.371-381.

Michel, A.L., 2002. Implications of tuberculosis in African wildlife and livestock. *Annals of the New York Academy of Sciences*, *969*(1), pp.251-255.

Muwonge, A., Johansen, T.B., Vigdis, E., Godfroid, J., Olea-Popelka, F., Biffa, D., Skjerve, E. and Djønne, B., 2012. *Mycobacterium bovis* infections in slaughter pigs in Mubende district, Uganda: a public health concern. *BMC Veterinary Research*, *8*(1), pp.1-7.

Pavlik, I., Ayele, W.Y., Havelkova, M., Svejnochova, M., Katalinic-Jankovic, V. and Zolnir-Dovc, M., 2003. *Mycobacterium bovis* in human population in four Central European countries during 1990–1999. *Veterinarni Medicina*, *48*(4), p.90.

Phepa, P.B., Chirove, F. and Govinder, K.S., 2016. Modelling the role of multi-transmission routes in the epidemiology of bovine tuberculosis in cattle and buffalo populations. *Mathematical Biosciences*, *277*, pp.47-58.

Ramdas, K.E., Lyashchenko, K.P., Greenwald, R., Robbe-Austerman, S., McManis, C. and Waters, W.R., 2015. *Mycobacterium bovis* infection in humans and cats in same household, Texas, USA, 2012. *Emerging infectious Diseases*, *21*(3), p.480.

Renwick, A.R., White, P.C.L. and Bengis, R.G., 2007. Bovine tuberculosis in southern African wildlife: a multi-species host–pathogen system. *Epidemiology & Infection*, *135*(4), pp.529-540.

© University of Pretoria

Rocha, V.C.F., Figueiredo, S.C.D., Rosales, C.A.R., Porto, C.D., Sequeira, J.L., Ferreira, J.S., Paes, A.C. and Salgado, V.R., 2017. Infection by *Mycobacterium bovis* in a dog from Brazil. *Brazilian Journal of Microbiology*, *48*, pp.109-112.

Rodríguez, S., Bezos, J., Romero, B., de Juan, L., Álvarez, J., Castellanos, E., Moya, N., Lozano, F., Javed, M.T., Sáez-Llorente, J.L. and Liébana, E., 2011. Mycobacterium caprae infection in livestock and wildlife, Spain. *Emerging Infectious Diseases*, *17*(3), p.532.

Sa'idu, A.S., Mohammed, S., Ashafa, M., Gashua, M.M., Mahre, M.B. and Maigado, A.I., 2017. Retrospective study of bovine tuberculosis in Gombe township abattoir, Northeastern Nigeria. *International Journal of Veterinary Science and Medicine*, *5*(1), pp.65-69.

Smith, N.H., Gordon, S.V., de la Rua-Domenech, R., Clifton-Hadley, R.S. and Hewinson, R.G., 2006. Bottlenecks and broomsticks: the molecular evolution of *Mycobacterium bovis. Nature Reviews Microbiology*, *4*(9), pp.670-681.

Sichewo, P.R., Hlokwe, T.M., Etter, E. and Michel, A.L., 2020. Tracing cross species transmission of *Mycobacterium bovis* at the wildlife/livestock interface in South Africa. *BMC Microbiology*, *20*(1), pp.1-9.

Stats, S.A., 2011. Statistics South africa. *Formal census*.

Warren, R.M., Gey van Pittius, N.C., Barnard, M., Hesseling, A., Engelke, E., De Kock, M., Gutierrez, M.C., Chege, G.K., Victor, T.C., Hoal, E.G. and Van Helden, P.D., 2006. Differentiation of Mycobacterium tuberculosis complex by PCR amplification of genomic regions of difference. *The International Journal of Tuberculosis and Lung Disease*, *10*(7), pp.818-822.

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Chapter 4. Review of data using the interferon-gamma assay for the detection of Mycobacterium *tuberculosis* complex spp. infections in livestock and game in South Africa available at the TB laboratory of OVR: Data for 6 years (2011-2016)

4 .1. Abstract

Background: In South Africa, the Agricultural Research Council-Onderstepoort Veterinary Research (ARC-OVR) is one of the laboratories that are DALRRD approved and SANAS accredited for the testing of bovine tuberculosis (bTB) using the IFN-γ assay. A retrospective study was conducted on the laboratory data at the Tuberculosis Laboratory for the period between 2011 and 2016. This study was conducted to estimate the occurrence of bTB across the nine provinces from which samples submitted to the Tuberculosis Laboratory for testing originated. Six years of data were collected and analysed to detect seropositivity using the Bovigam® kit (Bovidae species) and Primagam® for primates.

Results: Of the 10,369 samples tested, 1.54% (95% Confidence Interval: 1.30 – 1.78) were positive for bTB by serology using the Bovigam® kit. Avian reactors accounted for 3.75% (95% Confidence Interval: 3.38 - 4.11) of the samples tested while a combination of multiple and equal reactors as well as animals that had a positive screening test constituted 2.29% (95% Confidence Interval: 3.38 - 4.11). The distribution of the samples based on provinces ranged from Free State at 23.63% (95% Confidence Interval: 22.81 -24.45), Northern Cape at 18.25% (95% CI: 17.50 – 18.99), Gauteng at 9.68% (95% Confidence Interval: 9.11 – 10.25) with the least number of samples submitted for testing from the Western Cape at 0.49% (95% CI: 0.36 – 0.63). The study also revealed that out of 10,369 samples that were submitted and tested during the study period, the largest proportion tested were buffalo samples with 88.96% (95% CI: 88.35-89.56) followed by cattle at 9.94% (95% CI: 9.37 – 10.52).

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Conclusion: The study confirmed that bTB was endemic with low prevalence as previously mentioned. Buffaloes and cattle were the only species tested that were possibly exposed to *M. bovis* infection (positive, multiple or equal reactors). Although not statistically significant, animals originating from the Free State, KwaZulu-Natal, Limpopo and Mpumalanga were more likely to be infected or exposed. This study also demonstrated that serological data alone are insufficient to provide disease occurrence information in South Africa and it should be linked with bacteriological data to provide a better understanding of the disease occurrence in the country.

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4.2. Introduction

The causative agents for bovine tuberculosis (bTB) are members of the *Mycobacterium tuberculosis* complex (MTBC). *Mycobacterium bovis* and *M. tuberculosis* are two pathogens of the MTBC that are commonly isolated and are responsible for infecting livestock, wildlife, and humans (Brosch *et al.*, 2002; Ameni *et al.*, 2013). *Mycobacterium bovis* (*M. bovis*) is reported to have a wide host range, and reports on the isolation of other MTBC species such as *Mycobacterium mungi* (Alexander *et al.*, 2010) and *Mycobacterium orygis* (van Ingen *et al.*, 2012), have been documented. There have also been numerous reports on the isolation of non-tuberculous mycobacteria (NTM) and exposure to these mycobacteria results in cross-reactive immune responses, which hampers specificity of the interferon-gamma test (Michel, 2008; Gcebe & Hlokwe, 2017).

One of the most important strategies in the control of bTB is the early detection of infected animals (Schiller *et al.*, 2010). This is accomplished by detection of cellular immune responses of the infected animals using tests such as the IFN-γ (Buddle *et al.*, 2009). An experimental study in cattle by Buddle *et al.* (1994) revealed that cellular assays can detect infections as early as two weeks following a challenge with a dose of 500 cfu of *M. bovis*. Chamber *et al.* (2013) revealed that detection of tuberculosis using immunological tools remains a very important aspect in the diagnosis of TB, especially in surveillance programs.

A study by van der Heijden *et al.* (2016) compared cell-mediated and humoral immunity based assay for the detection of *M. bovis* infections in African buffaloes (*Syncerus caffer*) that were sampled in HiP in order to test the sensitivity. Out of 35 animals tested using Bovigam 1G, Bovigam 2G including the serological assay (IDEXX TB ELISA) tests results of the individual assays were compared. The ssensitivity of the Bovigam 1G, Bovigam 2G including the serological assay (IDEXX TB ELISA) were 100%, 75% and 37.5% respectively. The study

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concluded that the combination of the serological tests together with Bovigam 1G increases the chances of detection of infected animals (van der Heijden *et al.*, 2016)

Bovine tuberculosis is endemic in all nine provinces of South Africa and is a notifiable disease in South Africa. (Quirin *et al.*, 2001; Kahla *et al.*, 2011; Hlokwe *et al.*, 2014 ; Zahran *et al.*, 2014). The TB laboratory at the Agricultural Research Council-Onderstepoort Veterinary Research (ARC-OVR) is one of the laboratories designated for the serological testing for bTB using the interferon-gamma assay and record keeping.

This study aimed at reviewing laboratory data of six years (2011-2016) from the TB ARC-OVR laboratories. The data were livestock and game samples that were tested using the IFN-γ assay.

4.3. Materials and Methods

4.3.1. Study design

The study design was to collate and analyze laboratory data of samples that were tested for tuberculosis at the Tuberculosis laboratory at ARC-OVR, South Africa. Data abstraction for livestock and wildlife was collected from the laboratory books where all the information was recorded. The data set was arranged using Microsoft Excel version 2016 according to species, year, result, province, and sample description.

4.3.2 Study area

South Africa consists of nine provinces:- Eastern Cape, Western Cape, Northern Cape, Limpopo, Mpumalanga, North-West, KwaZulu-Natal, Free State, and Gauteng. The samples that were submitted for testing originated from all the provinces and were tested at the Tuberculosis laboratory (ARC-OVR) situated in Gauteng Province. The Tuberculosis laboratory is DALRRD approved and SANAS accredited in accordance with the requirements by the South African government for veterinary laboratories.

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4.3.3. Sampling

The laboratory data that were used in the study were retrieved from the records stored at the Tuberculosis Laboratory (ARC-OVR). Samples collected from livestock farms throughout South Africa, various wildlife species from KNP, HiP, and private game reserves were submitted for bTB serological testing between 2011-2016. All the samples were submitted together with a submission form that specified sample details such as the species of the animal tested, owner, and location (Hlokwe *et al*., 2014). The inclusion criteria were the following: (1) All public veterinary laboratory-generated results on tuberculosis spanning 2007 until 2016, (2) Suspected samples from domestic animals, wildlife, wild avian species, animals in water, and environmental samples, (3) Sample that originated within the territory of the Republic of South Africa, and (4) samples that were traceable epidemiological or by clinical history record to satisfy its inclusion. The exclusion criteria were (1) All public veterinary laboratory-generated results on tuberculosis outside the year range 2007 until 2016, (2) Samples processed in these laboratories above but originated from countries outside the Republic of South Africa, because of the designation of some of its laboratory as regional reference laboratories, and (3) All samples which were processed for tuberculosis diagnosis but did not have traceable epidemiological or clinical history record to satisfy its inclusion.

4.3.4. Laboratory tests data

All the samples were tested using a commercially purchased Bovigam® test kit (Prionics AG), for Bovidae, and Primagam® test kit (Prionics AG) for primates according to the manufacturer's instructions.

4.3.5. Data analysis

All data were entered and stored in Microsoft Excel version 2016. The retrospective data were analysed using Stata version 15 (StataCorp, College Station, TX, USA). Mean values were

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compared using a 95% confidence interval and the values were statistically significant when the p-value was < 0.05.

4.3.6. Ethical considerations

The study was approved by the Animal Ethics Committees for the Agricultural Research Council-Onderstepoort Veterinary Research (AEC 12.16) (Appendix 5.2) and the University of Pretoria (V104-17) (Appendix 5.3). Authorization to carry out the study at the abattoir was granted by the Department of Agriculture, Land Reforms and Rural Development (DALRRD) through section 20 approval (Appendix 5.4).

4.4. Results

4.4.1. Descriptive statistics of animal samples from 2011-2016

In this study, we collected laboratory data at the TB laboratory of the ARC-OVR for the period 2011-2016. During this time, 10,369 samples were submitted and tested using the Bovigam test® (bovidae) and Primagam for primates. The distribution of the samples based on the provinces ranged from Free State at 23.63% (95% CL: 22.81 -24.45), Northern Cape at 18.25% (95% CI: 17.50 – 18.99), Gauteng at 9.68% (95% CI: 9.11 – 10.25) and the least number of samples that was submitted for testing was from the Western Cape at 0.49% (95% CI: 0.36 – 0.63) the other 50% of the samples were obtained from the rest of the nine provinces as shown in Table 4.1 below .

Of the total number of samples tested from 2011-2016, the largest proportion was buffalo samples with 88.96% (95% Confidence Interval: 88.35-89.56) while 9.94% (95% Confidence Interval: 9.37 – 10.52) and 0.71% (95% Confidence Interval: 0.55 -0.88) were domestic bovine and Camelidae respectively. Antelope, caprine, eland, Oryx and wildebeest combined accounted for 2.31% (95% CI: 0.14 - 3.24) of the samples tested. Of the total number of

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samples, 92.42% (95% CI: 91.91 - 92.93) tested negative while 1.54% (95% CI: 1.30 – 1.78) tested positive, avian reactors were detected in 3.75% (95% CI: 3.38 – 4.11) of the samples as shown in Table 4.1. Equal reactors, multiple reactors, and sample with positive screening test results (a reaction during screening was considered positive if the bovine PPD sensitised samples yielded an optical density of ≥ 0.38) accounted for 2.29% (95% CI: 2.00 - 2.58). The majority of the samples were collected and tested in 2011 (25.81%; 95% CI: 24.97 - 26.65), see Table 4.1. Buffaloes and cattle were the only species possibly exposed to *M. bovis* infection (positive, multiple or equal reactors). A multiple reactor means an animal reacted positively to all tuberculin suggesting a possible infection with either *Mycobacterium bovis* or *Mycobacterium forfuitum* or both, and in some instances even *Mycobacterium avium*. An equal reactor means an animal reacted positively to both avian or bovine tuberculin due to possible infection with either *Mycobacterium bovis* or *Mycobacterium avium* or both.

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Table 4.1. Descriptive statistics of animal samples from South African provinces, 2011 – 2016.

Variable (n)	Category	Proportion (%)	$CI_{95\%}$
Species (10,369)	Antelope, caprine, eland, Oryx and wildebeest	2.31	0.14 - 3.24
	Buffalo	88.96	88.35 - 89.56
	Domestic bovine	9.94	9.37 - 10.52
	Camelidae	0.71	0.55 - 0.88
	Monkey, orangutan	0.14	0.07 - 0.22
Year of sample collection (10,369)	2011	25.81	24.97 - 26.65
	2012	14.94	14.25 - 15.63
	2013	19.45	18.69 - 20.21
	2014	14.03	13.36 - 14.70
	2015	16.13	15.43 - 16.84
	2016	9.57	9.00 - 10.13
	2017	0.04	< 0.001 - 0.07
	2018	0.03	< 0.004 - 0.06
Result (10,210)	Negative	92.42	91.91 - 92.93
	Positive	1.54	1.30 - 1.78
	Avian reactors	3.75	3.38 - 4.11
	Mixed infection*& doubtful	2.29	2.00 - 2.58
Province (10,369)	Eastern Cape	4.11	3.73 - 4.49
	Free State	23.63	22.81 - 24.45
	Gauteng	9.68	9.11 - 10.25
	KwaZulu Natal	0.71	0.55 - 0.88
	Limpopo	3.54	3.44 - 3.63
	Mpumalanga	1.94	1.67 - 2.20
	Northern Cape	18.25	17.50 - 18.99
	North West	5.83	5.37 - 6.28
	Western Cape	0.49	0.36 - 0.63
Sample description (10,369)	Heparinized blood	38.76	37.82 - 39.70
	Plasma	61.24	60.30 - 62.18

*Doubtful = Equal reactor, multiple reactor reactors, positive (high optical densities for samples sensitized with bovine PPD) but unclassified screening result. Statistical analyses were conducted and results were extracted using the Binomial Wald test.

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4.4.2. Multivariable logistics regression

Using samples from the Eastern Cape as a base, samples from the Northern Cape (OR: 0.24, 95% Cl: 0.08-0.69; $p < 0.01$), North West (OR: 0.21, 95% CI: 0.04-0.99; P=0.05), and Gauteng (OR: 0.22, 95%CI: 0.06-0.87; P=0.03) were less likely to be seropositive and statistical differences were significant (values were statistically significant when the p-value was < 0.05). Free State (OR: 1.41, 95%CI: 0.64-3.12; P=0.40), KwaZulu-Natal (OR: 1.92, 95%CI: 0.39-9.44; P=0.42), Limpopo (OR: 1.38, 95%CI: 0.63-3.02; P=0.41) and Mpumalanga (OR: 1.33, 95%CI: 0.38-4.59; P=0.45) were more likely to have positive animals but none of these provinces were statistically significant (values were statistically significant when the p-value was < 0.05). The year 2014 was more likely to produce positive animals and differences were statistically significant (OR: 2.29, 95%CI: 1.51-3.48; $p<0.001$) as shown in Table 4.2.

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Table 4.2. Univariable logistic regression to test the strength of association with risk of positivity of infection by Bovigam test.

Variable	Category	Odds Ratio	$CI_{95\%}$	*p*-value
Year	2011	1.00	NA	-
	2012	1.24	0.77 - 2.00	0.37
	2013	0.87	0.53 - 1.43	0.59
	2014	2.29	1.51 - 3.48	< 0.001
	2015	0.28	0.13 - 0.63	0.002
	2016	0.28	0.10 - 0.79	0.02
Province	Eastern Cape	1.00	NA	-
	Free State	1.41	0.64 - 3.12	0.40
	Gauteng	0.22	0.06 - 0.87	0.03
	KwaZulu Natal	1.92	0.39 - 9.44	0.42
	Limpopo	1.38	0.63 - 3.02	0.41
	Mpumalanga	1.33	0.38 - 4.59	0.45
	Northern Cape	0.24	0.08 - 0.69	< 0.01
	North West	0.21	0.04 - 0.99	0.05
	Western Cape	NA	NA	NA
Sample description	Heparinized blood	1.00	NA	-
	Plasma	1.06	0.76; 1.48	0.70

None of the species distribution predicted a higher risk or association with a positive outcome. NA = Not applicable.

4.5. Discussion

In South Africa, bTB is a controlled disease in all animal species with a comprehensive sustained control program and the provincial veterinary services are responsible for surveillance of the disease (Michel *et al.*, 2019). The results from the study show that majority of samples submitted for testing were buffalo samples. There are several reasons for the high number of buffalo samples submitted for testing. Firstly, buffaloes are not only the reservoir hosts for bTB, but they also have a huge impact on the transmission of other diseases such as foot and mouth (FMD) and brucellosis (Michel and Bengis, 2012). Buffaloes are a source of infection not only to other wild animals but to cattle as well (Miller, 2015), hence there is a need to monitor infections in these animal species. The other reason for the influx of buffalo

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samples could be as a result of the mandatory pre-movement testing of buffaloes in SA (Arnot & Michel, 2020) and that only test negative animals for TB, brucellosis and FMD are allowed to translocate to different regions (Hlokwe *et al*., 2016).

The results also revealed that the majority of the samples were submitted in 2011 and thereafter there was a decline in sample numbers. It was reported that the decentralization of the State Veterinary Services led to a drastic drop in the number of samples tested across all the provinces in South Africa (Arnot & Michel, 2020). The other reason may be due to insufficient funds available to sustain the surveillance programs (Michel *et al*., 2019). Also, 92.42% of the samples tested negative while 1.54% were positive. Except for sporadic outbreaks (Hlokwe *et al*., 2014) in the 1990s, a low prevalence of bovine tuberculosis (< 1.00%) has been reported and this was a result of a national bTB control programme that was implemented (Michel, 2008). Results obtained also show that approximately 2.29% of the samples were collected from animals that were possibly co-infected with *M. bovis* and non-tuberculous mycobacteria (i.e. equal and multiple reactors). Infection with NTMs (which are opportunistic pathogens) lowers the host immune system making it susceptible to infections with MTBC (Botha *et al*., 2013). Taking this factor into account, the cumulative sero prevalence will, however, still be low. The presence of avian reactors cannot be underestimated as NTM species cause cross-reactive immune responses not only in cattle but also wildlife species, and it is these immune responses that interfere with diagnostic assays such as the IFN-γ_ assay (Michel, 2008; Michel *et al*., 2011).

Dibaba & Kriek (2019) reported that South Africa is one of the few countries in Africa that have managed to sustain control programs. This could be one of the reasons why the occurrence of bTB has remained low. However, reports on bTB prevalence in communal cattle have produced seroprevalences ranging from 0.5%-15% in other studies conducted in SA (Musoke *et al*., 2015; Sichewo *et al*., 2019).

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The results also indicated that more samples were submitted from the Free State, but this was not statistically significant. This finding is in contrast with what was observed in the previous chapter (Chapter 3), where the majority of the samples for culture of mycobacterium species originated from Mpumalanga and were more likely to be positive (See Chapter 3). It should however, be noted that this study was based on a 10 year data analysis as compared to the current study which is based on a 6 year data analysis. A study by Hlokwe *et al* (2014), revealed that most of the samples submitted for culture had been collected from different livestock farms around South Africa including samples from (KNP) and (HiP) and private game ranches. The southern part of the KNP was reported to have a high disease prevalence, especially in wildlife, and currently, bTB is endemic in all nine provinces of South Africa (Hlokwe *et al*., 2014).

The current study revealed that samples (10,369) that were submitted in 2014 were more likely to be positive and the differences were significant ($p < 0.001$). The Department of Agriculture, Land Reforms and Rural Development (DALRRD) has indeed revealed that between the years 2000 and 2014, there were outbreaks that resulted in the culling of 16,881 cattle (Michel *et. al*., 2019), hence this could have attributed to the reason more samples were likely to test positive in 2014 as compared to other years when there was no outbreak.

4.6. Conclusion

The study confirmed that bTB is endemic with low prevalence in SA. Overall, the review of the 6-year data on tuberculosis in the samples tested using serological test data has demonstrated that serological data alone is insufficient for providing sufficient reporting on the prevalence of tuberculosis hence in the previous chapter a 10 year analysis on bacteriological data was conducted. A combination of both serological and bacteriological data would have

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an impact on policies for disease control in the country. Improved data collection and proper record keeping can significantly aid in the identification and management of the disease.

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4.7. References

Alexander, K.A., Laver, P.N., Michel, A.L., Williams, M., van Helden, P.D., Warren, R.M. and van Pittius, N.C.G., 2010. Novel *Mycobacterium tuberculosis* complex pathogen, *M. mungi*. *Emerging infectious diseases*, *16*(8), p.1296.

Ameni, G., Tadesse, K., Hailu, E., Deresse, Y., Medhin, G., Aseffa, A., Hewinson, G., Vordermeier, M. and Berg, S., 2013. Transmission of *Mycobacterium tuberculosis* between farmers and cattle in central Ethiopia. *PLoS One*, *8*(10), p.e76891.

Arnot, L.F. and Michel, A., 2020. Challenges for controlling bovine tuberculosis in South Africa. *Onderstepoort Journal of Veterinary Research*, *87*(1), pp.1-8.

Botha, L., Gey van Pittius, N.C. and Van Helden, P.D., 2013. Mycobacteria and disease in southern Africa. *Transboundary and Emerging Diseases*, *60*, pp.147-156.

Brosch, R., Gordon, S.V., Marmiesse, M., Brodin, P., Buchrieser, C., Eiglmeier, K., Garnier, T., Gutierrez, C., Hewinson, G., Kremer, K. and Parsons, L.M., 2002. A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. *Proceedings of the national academy of Sciences*, *99*(6), pp.3684-3689.

Buddle, B.M., Aldwell, F.E., Pfeffer, A., Lisle, G.D. and Corner, L.A., 1994. Experimental *Mycobacterium bovis* infection of cattle: effect of dose of M. bovis and pregnancy on immune responses and distribution of lesions. *New Zealand Veterinary Journal*, *42*(5), pp.167-172.

Buddle, B.M., Livingstone, P.G. and De Lisle, G.W., 2009. Advances in the ante-mortem diagnosis of tuberculosis in cattle. *New Zealand Veterinary Journal*, *57*(4), pp.173-180.

Chambers, M.A., 2013. Review of the diagnosis of tuberculosis in non-bovid wildlife species using immunological methods–an update of published work since 2009. *Transboundary and Emerging Diseases*, *60*, pp.14-27.

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Dibaba, A.B. and Kriek, N.P., 2019. The Control of Bovine Tuberculosis in Africa. In *Tuberculosis in Animals: An African Perspective* (pp. 237-270). Springer.

Gcebe, N. Hlokwe, T. M. 2017. Non-tuberculous mycobacteria in South African wildlife: Neglected pathogens and potential impediments for bovine tuberculosis diagnosis *Frontiers in Cellular and Infection Microbiology* 7:1-5.

Hlokwe, T.M., Van Helden, P. and Michel, A.L., 2014. Evidence of increasing intra and inter-species transmission of *Mycobacterium bovis* in South Africa: are we losing the battle? *Preventive Veterinary Medicine*, *115*(1-2), pp.10-17.

Hlokwe, T.M., De Klerk-Lorist, L.M. and Michel, A.L., 2016. Wildlife on the move: a hidden tuberculosis threat to conservation areas and game farms through introduction of untested animals. *Journal of Wildlife Diseases*, *52*(4), pp.837-843.

Kahla, I.B., Boschiroli, M.L., Souissi, F., Cherif, N., Benzarti, M., Boukadida, J. and Hammami, S., 2011. Isolation and molecular characterisation of *Mycobacterium bovis* from raw milk in Tunisia. *African Health Sciences*, *11*, pp.2-5.

Michel, A. 2008. *Mycobacterium fortuitum* infection interference with *Mycobacterium bovis* diagnostics: natural infection cases and a pilot experimental infection. *Journal of Veterinary Diagnostic Investigation* 20:501-503.

Michel, A.L., Cooper, D., Jooste, J., De Klerk, L.M. and Jolles, A., 2011. Approaches towards optimising the gamma interferon assay for diagnosing Mycobacterium bovis infection in African buffalo (Syncerus caffer). *Preventive veterinary medicine*, *98*(2-3), pp.142-151.

Michel, A.L. and Bengis, R.G., 2012. The African buffalo: a villain for inter-species spread of infectious diseases in southern Africa. *Onderstepoort Journal of Veterinary Research*, *79*(2), pp.26-30.

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Michel, A.L., Sibanda, D.R. and de Klerk-Lorist, L.M., 2019. BTB Control Strategies in Livestock and Wildlife in South Africa. In *Tuberculosis in animals: An African perspective* (pp. 387-401). Springer, Cham.

Miller, M., White, P.C. and Bengis, R.G., 2015. Tuberculosis in South African wildlife: why is it important? *Sun Media: Stellenbosch, South Africa*.

Musoke, J., Hlokwe, T., Marcotty, T., Du Plessis, B.J. and Michel, A.L., 2015. Spillover of *Mycobacterium bovis* from wildlife to livestock, South Africa. *Emerging infectious diseases*, *21*(3), p.448.

Schiller, I., Vordermeier, H.M., Waters, W.R., Whelan, A.O., Coad, M., Gormley, E., Buddle, B.M., Palmer, M., Thacker, T., McNair, J. and Welsh, M., 2010. Bovine tuberculosis: effect of the tuberculin skin test on in vitro interferon gamma responses. *Veterinary Immunology and Immunopathology*, *136*(1-2), pp.1-11.

Sichewo, P.R., Michel, A.L., Musoke, J. and Etter, E., 2019. Risk factors for zoonotic tuberculosis at the wildlife–livestock–human Interface in South Africa. *Pathogens*, *8*(3), p.101.

Van der Heijden, E.M.D.L., Jenkins, A.O., Cooper, D.V., Rutten, V.P. and Michel, A.L., 2016. Field application of immunoassays for the detection of Mycobacterium bovis infection in the African buffalo (Syncerus caffer). *Veterinary immunology and immunopathology*, *169*, pp.68-73.

van Ingen, J., Rahim, Z., Mulder, A., Boeree, M.J., Simeone, R., Brosch, R. and van Soolingen, D., 2012. Characterization of *Mycobacterium orygis* as *M. tuberculosis* complex subspecies. *Emerging infectious diseases*, *18*(4), p.653.

Quirin, R., Rasolofo, V., Andriambololona, R., Ramboasolo, A., Rasolonavalona, T., Raharisolo, C., Rakotoaritahina, H., Chanteau, S. and Boisier, P., 2001. Validity of intradermal

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tuberculin testing for the screening of bovine tuberculosis in Madagascar. *Onderstepoort Journal of Veterinary Research*, 68(3), pp. 231

Zahran, R.N., El Behiry, A., Marzouk, E. and Askar, T., 2014. Comparison of LCD array and IS6110-PCR with conventional techniques for detection of *Mycobacterium bovis* isolated from Egyptian cattle and Buffaloes. *International Journal of Mycobacteriology*, *3*(3), pp.197-204.

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Chapter 5. The knowledge, attitude, practice (KAP) and the risk of exposure of abattoir workers to *Mycobacterium* spp. in Gauteng Province, South Africa

5.1. Abstract

Background: Zoonotic tuberculosis (TB) remains a global public health hazard. Information based on knowledge, practices, and risk factors remains crucial, especially among high-risk occupations such as veterinarians and abattoir workers**.** The purpose of this study was to examine the knowledge, attitude, and practice (KAP) to assess the risk of exposure to *Mycobacterium* spp., by abattoir workers in Gauteng province of South Africa.

Materials and methods: A close-ended questionnaire was used to interview 103 abattoir workers from six different abattoirs between March and May 2018. Data relating to their knowledge and risks of exposure to *Mycobacteria* were collected from the abattoir workers. The data were analyzed using Statistical Package Stata 14 (StataCorp, College Station, TX U.S.A.) for descriptive analysis. Tables and bar charts were constructed using Microsoft Excel 2010.

Results: A total of 103 abattoir workers participated in the study, with more males than females (87/103: 84.47%). Majority of the participants (80.58%, 83/103) worked in the slaughter and meat processing sections of the abattoirs. High proportions (88.35%) of the participants were found to be knowledgeable about zoonotic tuberculosis. The study also revealed the respondents overall knowledge score of 42% (95% Confidence Interval: 37.48 - 46.42, $p<0.001$). Analysis showed that approximately 45% (95% Confidence Interval: 35.28 – 56.02) of the participants were aware that they could be infected by animals and 44.6% (95% Confidence Interval: 34.22 – 54.91) were aware that they could transmit tuberculosis to their animals. Only a few of the respondents (10.68% and 25.24%) consumed undercooked/raw meat

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and consumed unpasteurized milk, respectively, hence, the risk of TB transmission via ingestion was found to be relatively low. Overall, participants were aware that humans can transmit tuberculosis to animals (P = 0.003).

Discussion and conclusion: The findings revealed the practices that have the potential risk to promote tuberculosis transmission from animals to abattoir workers and amongst the workers, demonstrating the need to introduce programmes that address occupational exposure, prevent, and control of bovine tuberculosis (bTB) at abattoirs. A high proportion of abattoirs workers demonstrated good practice by having and wearing personnel protective equipment (PPE) and this practice minimizes the transmission or spread of diseases such as tuberculosis. However, a sustained training programme for abattoir workers on risk factors leading to transmission of tuberculosis and other zoonoses is recommended amongst the workers and their communities. Information about employee perception of tuberculosis could not be obtained because the questionnaire did not contain questions focussed on this aspect.

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5.2 Introduction

Tuberculosis (TB) remains an important disease, particularly in Africa where it represents a huge threat to public health and threatens international trade (Ayele *et al.*, 2004). The presence of *Mycobacterium bovis* at the human-livestock-wildlife interface confirms its significance as a zoonotic disease (Ogudenji *et al.*, 2015). Human and animal tuberculosis are reported to be caused by *Mycobacterium tuberculosis* and *M. bovis* respectively, with genetic similarities (Rogal *et al.*, 1990). *Mycobacterium bovis* is responsible for zoonotic TB and as a result, in 2015, approximately 147 000 new zoonotic TB cases with 12,500 deaths were recorded worldwide with the highest incidence recorded on the African continent (Raviglione & Korobitsyn, 2016).

There are no accurate data available on zoonotic tuberculosis so it is impossible to estimate the true disease burden, an indication that cases of humans contracting zoonotic tuberculosis might be higher than estimated. Additionally, published data normally do not represent zoonotic tuberculosis nationally, but rather focus on selected groups (Olea-Popelka *et al.*, 2017). Furthermore, the risk of transmission increases in TB- endemic areas where humans, such as veterinarians, and abattoirs workers come into direct contact with infected animals (Cosivi *et al.*, 1998), or animal products such as unpasteurized milk (Michel *et al.*, 2015).

In humans, *M. bovis* is transmitted by consumption of raw or undercooked meat, consumption of unpasteursized milk, or inhaling contaminated droplets from infected animals (Cosivi *et al.*, 1998; Miller, 2015). Considering that *M. bovis* has been isolated from lesions of different organs and tissues from animals slaughtered at abattoirs shows that the disease can spread through direct and indirect routes to humans (de la Rua-Domenech, 2006; LoBue *et al.*, 2010;). Vayr *et al.* (2018) reported that abattoir workers are at risk from occupational exposure as they

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might come across infected carcass. This poses a huge transmission risk concern for farmers, veterinarians and slaughterhouse workers (LoBue *et al*., 2010).

Effective practices for preventative zoonotic TB are of utmost importance, especially among high-risk occupations such as abattoir workers and veterinarians. A study on TB knowledge and practices can indicate where gaps exist in zoonotic disease among animal handlers. There are no accurate data available on zoonotic tuberculosis in the country therefore, it is impossible to estimate the true disease burden, an indication that cases of humans contracting zoonotic tuberculosis might be higher than estimated. Additionally, published data normally do not represent zoonotic tuberculosis nationally, but rather focus on selected groups. A study on TB knowledge and practices can indicate where gaps exist in zoonotic disease among animal handlers. Overall, the knowledge and practices of zoonotic TB are poorly investigated in occupationally exposed individuals (Adesoka *et al*., 2015). Thus, there is limited data available on zoonotic data nationally, especially on abattoirs. Hence, the study aimed to analyze the knowledge, attitude, and practice (KAP) to *Mycobacterium* spp. from red meat abattoir workers in Gauteng province of South Africa. Additionally, we aimed to assess the risk of exposure of abattoir workers to *Mycobacterium* spp. including *Mycobacterium tuberculosis* complex bacteria causing tuberculosis.

5.3. Materials and methods

5.3.1. Study area

The study was conducted at red meat abattoirs in Gauteng province, South Africa. Although Gauteng is the smallest province in SA with an area of approximately 18 178km^2 it is the most populous province and it is considered an economic engine of the country (Figure 5.1). It consists of six municipal districts, i.e., the City of Johannesburg, the City of Tshwane, Ekurhuleni, Metsweding, Sedibeng and West Rand. The study was conducted at 6 abattoirs

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that were located in these districts: 1) ARC-Irene and 2) Klipeiland abattoirs located in the City of Tshwane district; 3) Chamdor abattoir located in the West Rand district; 4) Comet abattoir located at Ekurhuleni district; 5) Bochkop and 6) Rietspruit abattoirs located at Sedibeng districts (Figure 5.1).

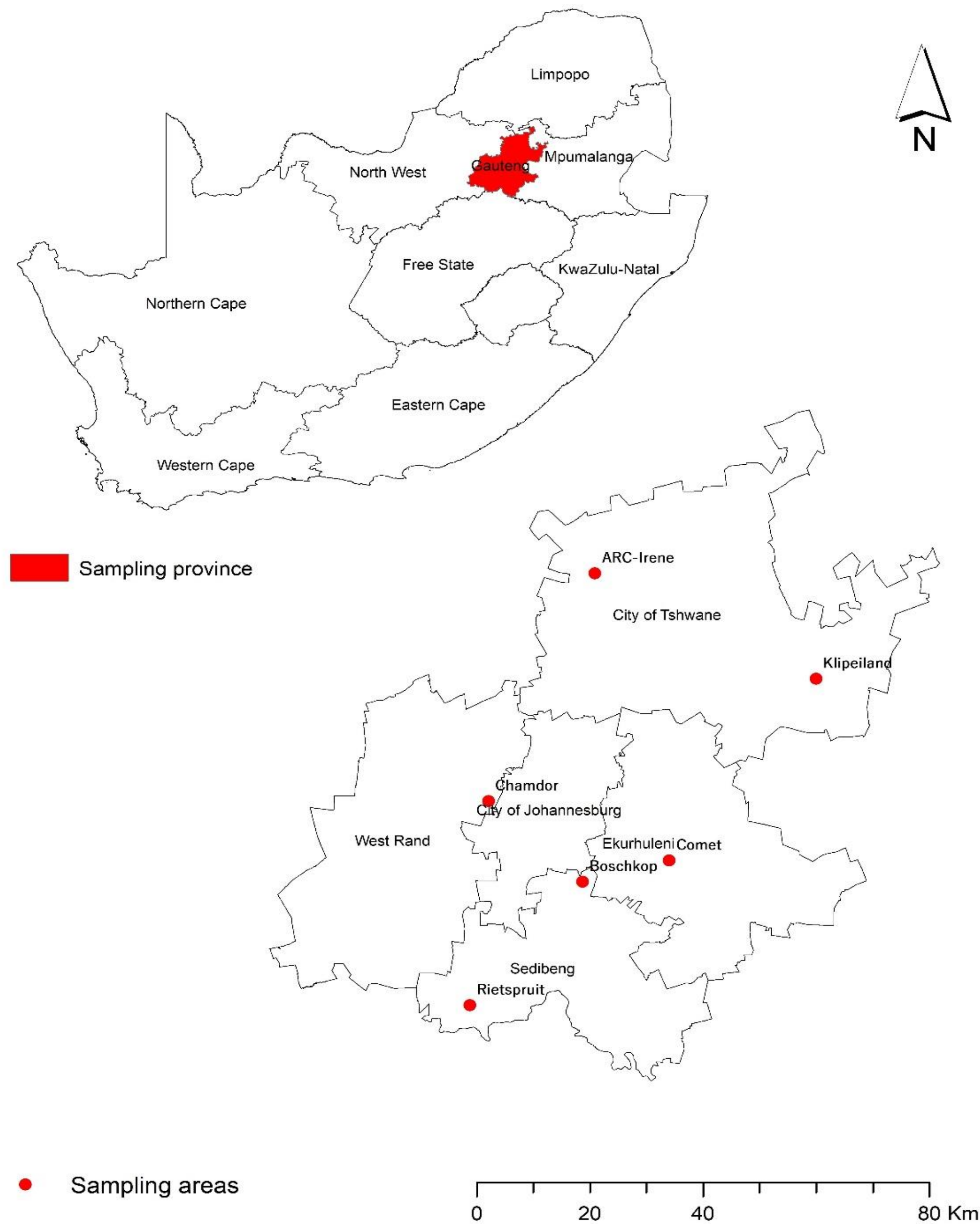

Figure 5.1: Map showing the location of the red meat abattoirs in Gauteng province where the questionnaires were administered.

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5.3.2. Study design and questionnaire

A close-ended questionnaire (Appendix 1) was prepared and administered to 103 abattoir workers from six different abattoirs between March and May 2018. The questionnaire was pre-tested, and all the necessary changes were made before the study commenced. We selected workers from both high and low-throughput abattoirs. Participating abattoir workers were chosen based on random sampling and their willingness to participate. The questionnaire was developed to assess the knowledge, attitude, and practices of abattoir workers that could predispose them to tuberculosis.

5.3.3. Data collection

The questionnaire was divided into five sections; the first section consisted of socio-demographic information including age, gender, marital status, occupation, and experience. The second section focused on practices that abattoir workers engage in that may expose them to infection with *Mycobacterium* spp. species that cause TB. These included questions such as if workers have animals at home, if they consume unpasteurized milk and undercooked or raw meat, if they slaughter animals at home, and if they use personal protective gear at home. The third section questioned their knowledge of TB, if they were diagnosed with TB, if they knew they could TB from animals or if they knew that they could transmit the infection to their animals. The fourth section consisted of symptoms they experienced while working in the abattoir and the last section was where they obtain drinking water in the abattoirs and at home and how water is purified at home and at the abattoir. Verbal and written consent was sought before the interviews were conducted. The questionnaire was developed in English and administered mostly in the local language that is spoken and understood by respondents and the person administering the questionnaire was conversant in the local language.

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5.3.4. Statistical analysis

Data were analyzed using Statistical Package Stata 14 (StataCorp, College Station, TX U.S.A.) for descriptive analysis. Tables and bar charts were constructed using Microsoft Excel 2010. Values of $p<0.05$ were regarded as statistically significant. Data were presented as frequencies and percentages. The data obtained from the study were subjected to univariable analysis to generate the 95% CI.

5.3.5 Ethical considerations

The study was approved by the Animal Ethics Committees for the Agricultural Research Council-Onderstepoort Veterinary Research (AEC 12.16) (Appendix 5.2) and the University of Pretoria (V104-17) (Appendix 5.3). Authorization to carry out the study at the abattoir was granted by the Department of Agriculture, Land Reforms and Rural Development (DALRRD) through section 20 approval (Appendix 5.4).

5.4. Results

5.4.1. Socio-demographic information

A total of 103 abattoir workers participated in the study, including management (n=17) and general abattoir workers (n=86). There were more male (84.47%, 87/103) than female respondents (15.53%, 16/103). The majority of respondents (41.75%, 43/103) were between the ages of 26-35 years. Also, a large number of the respondents (80.58%, 83/103) worked in the slaughter and meat processing sections of the abattoirs as compared to the loader or transporter section. The socio-demographic features are shown in Table 5.1.

5.4.2. Knowledge of tuberculosis by abattoir workers

Knowledge of TB among abattoir workers was high at 88.35% (95% Confidence Interval: 82.04 – 94.65). When asked about the zoonotic implications, more than 45% (95% Confidence

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Interval: 35.28 – 56.02) of abattoir workers knew that they could be infected with *M. bovis* from infected animals, while 44.6% (95% Confidence Interval: 34.22 – 54.91) were aware that they could transmit TB to animals if they are infected with the TB causing pathogens. A low percentage of the respondents that is 7% (95% Confidence Interval: 2.09-13.13) were diagnosed to have TB previously (Table 5.2).

Table 5.1: Socio-demographic features of the survey respondents from the different districts in the Gauteng Province.

Variable name	Category	Frequency (n=103)	Percentage (%)
Age group (years)	18-25	14	13.59
	26-35	43	41.75
	36-50	33	32.04
	51-60	13	12.62
Gender	Female	16	15.53
	Male	87	84.47
Marital Status	Single	61	59.22
	Married	42	40.78
Occupation (Job)	Hide processor	1	0.97
	Loader	1	0.97
	Management	17	16.50
	Slaughter and Processing	83	80.58
	Transporter	1	0.97
How long have you worked at the abattoir?	1 year	21	20.39
	2 years	17	16.50
	3years	64	62.14
	4 years	1	0.97

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Table 5.2. Participants' responses to questions of their knowledge of zoonotic TB and its transmission

Variable	Category	(%) Percentage Frequency	95%CI
Do you know what TB is?	Yes	88.35 (91/103)	82.04 – 94.65
Have you ever been sick from TB?	Yes	7.6 (7/92)	2.09 – 13.13
Has any member of your family been sick from TB before?	Yes	18.2 (17/93)	10.28 – 26.28
Do you think you can get TB from animals?	Yes	45.7 (42/92)	35.28 – 56.02
Do you think you can infect animals with TB?	Yes	44.6 (41/92)	34.22 – 54.91

Practices that could promote tuberculosis infection were identified as taking care of animals at home or working at the abattoir, consumption of unpasteurized milk, consumption of or raw meat, slaughtering animals at home, whether they have or wear personal protective gear, and whether workers were vaccinated against TB. Bad practices are those that abattoir workers engage in that could promote tuberculosis infection and transmission. Whereas good practices include the consumption of pasteurized milk, consumption of cooked meat, and wearing personal protective gear during slaughter. Some of the respondents engaged in practices that put them at risk of infection as 35.92% (95% CI; 26.50- 45.34) indicated that they take care of animals either at their homes, work (abattoir) or their farms, while 47.57% (95 Confidence Interval; 37.76-57.38) of the respondents mentioned that they slaughtered animals at home. Furthermore, 10.68% (95 Confidence Interval: 4.61-16.75) and 25.24% (95% Confidence Interval: 16.71 –33.77) of the respondents indicated consumption of undercooked or raw meat and consumed unpasteurized milk respectively. Results showed that the majority (95.15%) of the participants spend most of their time (5-7 days) at work. Additionally, most of the abattoir

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workers demonstrated a good practice of prevention of zoonotic TB as 94.17% (95% Confidence Interval: 89.57-98.77) have personal protective gear and 95.15% (95% Confidence Interval: 90.92–99.37) wear their protective gear (Table 5. 3).

Table 5.3: Practices that may promote Tuberculosis infection by abattoir and abattoir workers

Variable	Category	Percentage Frequency (n=103)	95%CI
Do you take care of animals at home, work or farm?	Yes	35.92 (32/103)	**26.50- 45.34**
If yes, which types of animals?	• Livestock • Pets • Wildlife	67.74 (21/103) 25.81 (8/103) 6.45 (2/103)	**-44.50- 111.20**
How many days do you work in the abattoir per week?	• 1-2 days per week • 3-4 days per week • 5-7 days per week	2.91 (3/103) 1.94 (2/103) 95.15 (98/103)	**-99.6 - 166.2**
Do you consume unpasteurized milk?	Yes	25.24 (26/103)	**16.71 –33.77**
Do you consume uncooked or undercooked meat?	Yes	10.68 (11/103)	**4.61-16.75**
Do you slaughter animals at home?	Yes	47.57 (49/103)	**37.76-57.38**
Do you have personal protective gear?	Yes	94.17 (97/103)	**89.57-98.77**
Do you wear personal protective gear?	Yes	95.15 (98/103)	**90.92–99.37**
Have you been vaccinated against TB?	Yes	50.00 (46/103)	**39.59-60.41**

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5.4.4. Signs and symptoms experienced by abattoir workers while working in the abattoirs

Out of the 103 respondents interviewed, 79.6% reported to have experienced symptoms such as fever, cold, loss of appetite, cough, body pain, night sweats, and weakness. Table 5.4 shows that 53.4% of the respondents experienced cold and 47.57% coughed while working in the abattoir.

Table 5.4: Signs and symptoms experienced by abattoir workers while working in the abattoir

Variable	Category	Percentage Frequency	95%CI
Have you experienced these symptoms working in the abattoir?	Yes	79.6 (82/103)	72.0 – 87.52
Fever	Yes	42.72 (44/103)	33.0 – 52.43
Cold	Yes	53.40 (55/103)	43.6 – 63.20
Loss of appetite	Yes	34.95 (36/103)	25.59 – 44.32
Cough	Yes	47.57 (49/103)	37.76 – 57.38
Body pain	Yes	39.81 (41/103)	30.19 – 49.42
Night Sweat	Yes	30.10 (31/103)	21.10 – 39.11
Weakness	Yes	19.42 (20/103)	11.65 – 27.19

5.5 Multivariable associations

5.5.1 Association between age group and symptoms the respondents experienced while working in abattoirs

Results revealed that age was not significantly associated with most of the symptoms experienced by participants while working in the abattoir. Night sweats was the only symptom that was found to be statistically significantly different among all age groups (p = 0.021) as shown in Table 5.5 below.

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Table 5.5: Relationship between age group and symptoms the respondents experienced while working in abattoirs

	No. (%) with symptoms in different age groups:				
Symptom	**18-25**	**26-35**	**36-50**	**51-60**	**p-value**
Have you experienced symptoms while working in the abattoir?	14 (92.86)	43 (76.74)	33 (84.85)	13 (61.54)	0.215
Fever	14 (57.14)	43 (41.86)	33 (45.45)	13 (23.08)	0.357
Cold	14 (57.14)	43 (46.51)	33 (66.67)	13 (38.46)	0.228
Loss of appetite	14 (35.71)	43 (39.53)	33 (36.36)	13 (15.38)	0.474
Cough	14 (57.14)	43 (48.84)	33 (54.55)	13 (15.38)	0.082
Body Pains	14 (57.14)	43 (39.53)	33 (42.42)	13 (15.38)	0.167
Night Sweat	14 (7.14)	43 (23.26)	33 (48.48)	13 (30.77)	0.021
Weakness	14 (21.43)	43 (23.26)	33 (18.18)	13 (7.69)	0.734

5.5.2. Association between participants' age group and having knowledge of TB

There was no significant association between age and knowledge of TB (p=0.885) as shown in Table 5.6 below.

Table 5.6: Relationship between participants' age group and having knowledge of TB

	No. (%) with knowledge of TB in different age groups:				
Age Group	**18-25**	**26-35**	**36-50**	**51-60**	**p-value**
Do you know about TB?	14 (13.19)	43 (40.66)	33 (32.97)	13 (13.19)	0.885

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5.5.3. Association between knowledge of TB with gender or marital status

There was no significant association between knowledge of TB and gender (*p* = 0.409). In addition, there was no association between knowledge of TB and marital status (*p* = 0.409) as demonstrated in Table 5.7 below.

Table 5.7: Relationship between knowledge of TB with gender or marital status

	Knowledge of TB		
Gender	**Frequency**	**Percentage**	**p-value**
Female	16/103	16.48	0.409
Male	87/103	83.52	
Marital Status	**Frequency**	**Percentage**	**p-value**
Married	42/103	41.76	0.409
Single	61/103	58.24	

5.5.4. Association between age group vs having been sick with TB and knowledge that humans can infect animals with TB

A statistically significant association between the age group and respondents being sick with TB was observed, P = 0.009, (Table 5.8). In addition, the results obtained show a statistically significant association between age group and knowledge that humans can infect animals with TB (P = 0.003) as shown in Table 5.8 below.

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Table 5.8: Relationship between age group vs having been sick with TB and knowledge that humans can infect animals with TB

	Have you ever been sick with TB?		
	Frequency	**Percentage**	**p-value**
Age group			
18-25	12	0.00	0.009
26-35	37	0.00	
36-50	31	57.14	
51-60	12	42.86	
	Humans can infect animals		
	Frequency	**Percentage**	**p-value**
Age group			
18-25	12	9.76	0.030
26-35	37	39.02	
36-50	31	26.83	
51-60	12	24.39	

5.5.9. Association between different water sources in the abattoir versus symptoms experienced while working

Water sources were investigated for their role in posing a risk of infection to abattoir workers. However, there was no association between water source and symptoms experienced while working in the abattoir (P=0.810) as shown in Table 5.9.

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Table 5.9: Association of different water sources in the abattoir versus symptoms experienced while working in the abattoir with symptoms of tuberculosis.

Symptom	Dam\Well	Other	Tap	p-value
Cough	7 (58.33)	7 (43.75)	35 (46.67)	0.810
Night Sweat	4 (33.33)	3 (18.75)	24 (32.00)	0.577
Cold	5 (41.67)	7 (43.75)	43 (57.33)	0.475
Loss of appetite	3 (25.00)	5 (31.25)	28 (37.33)	0.753
Fever	3 (25.00)	5 (31.25)	36 (48.00)	0.245
Body Pains	3 (25.00)	6 (37.50)	32 (42.67)	0.518
Weakness	1 (8.33)	5 (31.25)	14 (18.67)	0.306

5.5.10. Associations between age group and members of the family being sick with TB and knowledge that people can contract TB from animals, and the participants' vaccination history.

Both age groups 26-35 and 36-50 had the highest number of respondents that had family members who were previously infected with TB (35.29%). However, these variables were not statistically significant (P = 0.484). Participants in the age group 26-35 years highlighted that they were aware of the zoonotic nature of TB (40.48%). However, these variables were not statistically significant (P = 1.000). Although age groups 51-60 of respondents were vaccinated against TB (66.67%), these variables were not statistically significant (P = 0.439) as shown in Table 5.10.

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Table 5.10: Relationship between age group and sickness of family members with TB, knowledge that people can contract TB from animals as well as participants' vaccination history.

	Family diagnosed with TB		
Age group	**Frequency**	**Percentage**	**p-value**
18-25	12	23.53	0.484
26-35	38	35.29	
36-50	31	35.29	
51-60	12	5.88	
	Knew that TB can be transmitted from animals to humans		
Age group	**Frequency**	**Percentage**	**p-value**
18-25	12	11.90	1.000
26-35	37	40.48	
36-50	31	33.33	
51-60	12	14.29	
	Vaccinated against TB		
Age group	**Frequency**	**Percentage**	**p-value**
18-25	12	33.33	0.439
26-35	37	51.35	
36-50	31	48.39	
51-60	12	66.67	

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5.6. Association between age group, consumption of unpasteurized milk, of uncooked or undercooked meat, slaughtering of livestock at home and having personal protective gear

5.6.1. Association between age group and frequency (%) consumption of unpasteurized milk

Findings show that the age group of 36-50 years (33.3%) had the highest number of respondents that consumed unpasteurized milk as compared to the age group 51-60 which had the least number of respondents consuming unpasteurized milk (15.4%). The differences were not statistically significant (*p*=0.653), figure 5.2.

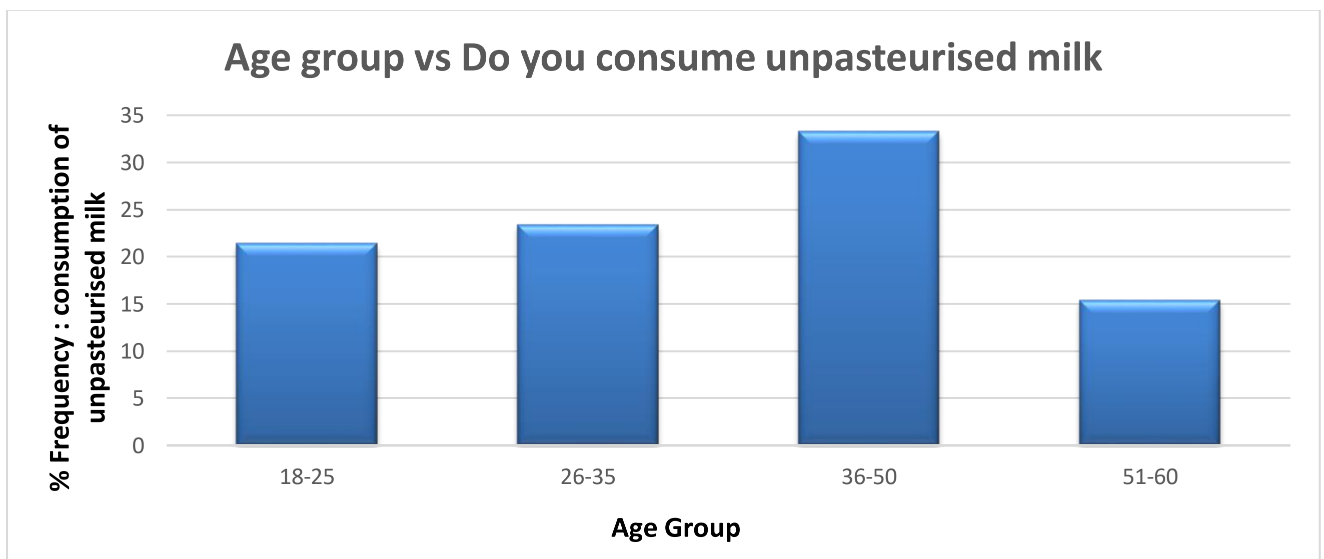

Figure 5.2: Relationship between age group and frequency (%) consumption of unpasteurized milk.

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5.6.2. Association between age group and the frequency (%) of consumption of uncooked or undercooked meat.

Results show that the age group of 51-60 years had the highest frequency of respondents (23.08%) who consumed uncooked or undercooked meat as shown in Figure 5.3 below. There was no statistically significant differences (P=0.393).

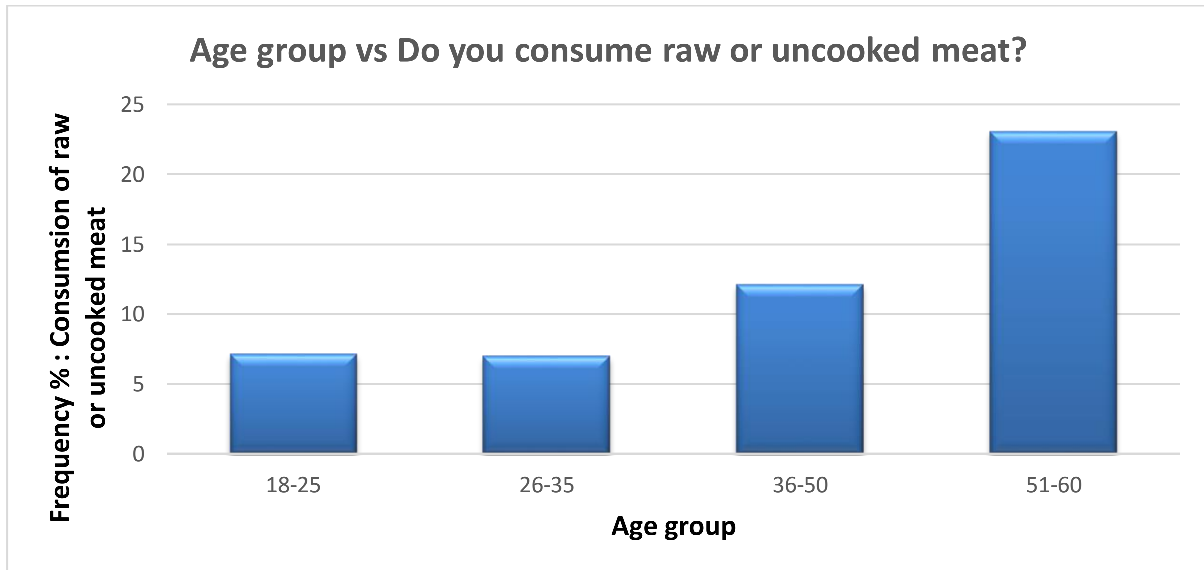

Figure 5.3: Association between age group and the frequency (%) of consumption of uncooked or undercooked meat.

5.6.3. Association between age group and frequency (%) of slaughtering of livestock at home.

Results show that age group of 36-50 years (60.61%) had the highest number of respondents that slaughtered livestock at home (Figure 5.4). However, there was no significant differences (P=0.313) among the age groups.

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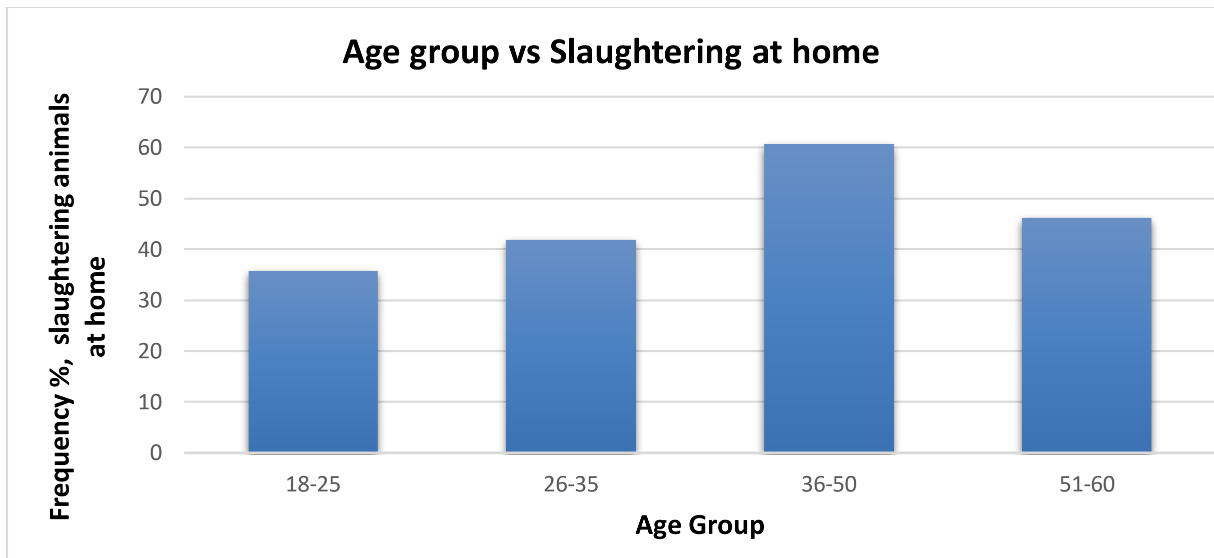

Figure 5.4: Relationshipbetween age group and frequency (%) of slaughtering of livestock at home.

5.6.4. Association between the age group of abattoir workers and frequency of having personal protective gear.

All (100.0%) the people in two age groups (18-25 and 51-60) had personal protective gearcompared to 88.37% for workers in the 26-35 years age group (Figure 5.5). There was no significant differences (P=0.316).

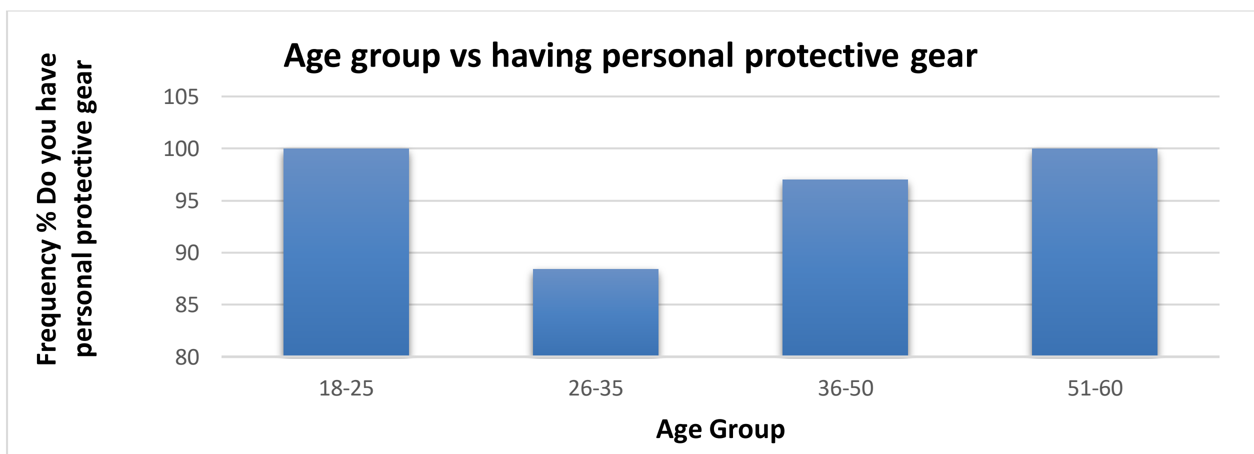

Figure 5.5: Relationship between the age group of abattoir workers and frequency of having personal protective gear.

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5.7 Discussion

The current study aimed at assessing the KAP using data collected from abattoir workers in Gauteng province, SA, to assess the risk of exposure of abattoir workers to *Mycobacterium* spp. The majority of the respondents in this study were male 87.47% (87/103) and young adults between the ages 26-35 (43/ 103). This characteristic was also observed in a previous study (Ismaila *et al*., 2015), where the majority of the respondents were young adults. This is probably attributed to the fact of young adults are able-bodied and have the physical strength that abattoir settings require (Ismaila *et al*., 2015).

The current study found that over 80% of the respondents knew about TB, a frequency much higher than found in a previous study conducted in countries such as Nigeria. For example, a study by Kachalla *et al.* (2015) found that 53.2% of the abattoir workers from two Abuja abattoirs in Nigeria had a fair knowledge of the disease. Another study by Ismaila *et al.* (2015) from the same country, reported a low level of 46.3% for participants with knowledge of bovine tuberculosis by participants. In SA, Marange *et al.* (2020) reported the bTB knowledge of participants at 61% which is lower than observed in the current study. However, the previous study (Marange *et al.,* 2020) was conducted in the pastoral community while our study is abattoir-based. A possible reason for the high levels of knowledge of zoonotic TB observed in the current study may be due to the following factors: the research team made both oral and written presentations on zoonotic diseases such as tuberculosis and brucellosis, during earlier visits to the same abattoirs to collect samples from slaughtered livestock, The fact that the presentations were conducted six months before interviews, thus making abattoir workers aware of the diseases. It is important to note that a low level of knowledge presents a huge concern and a public health concern as abattoir workers are in a high disease risk occupation.

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The study shows that 45.7% (95% Confidence Interval: 35.28-56.02) of the respondents were aware that the disease can be transmitted from animals to humans and 44.6% (95% Confidence Interval: 34.22-54.91) were aware that the disease could also be transmitted from humans to animals. This is significant because less than 50% of the abattoir workers were aware that TB is a zoonosis. The lack of awareness is considered a factor that could predispose abattoir workers to infections, as they are unaware of the zoonotic transmission of TB. Datiko *et al*. (2019) noted that lack of awareness is a risk factor as it results in the transmission of the disease. In this study, several factors associated with bovine tuberculosis such as taking care of animals at home/work, consumption of unpasteurized milk, uncooked or raw meat, and the slaughter of animals at home, were identified as factors that promote transmission of TB. One of the practices that predispose the respondents to bovine tuberculosis is the consumption of unpasteurized milk and undercooked or raw meat. The study shows that 25.24% of the respondents consumed unpasteurized milk and 10.68% consumed undercooked/raw meat. This finding is comparable to findings in a study by Mohammed *et al*. (2019) in Nigeria who reported 24.1% of participants consumed unspateurised milk. However, the same study by Mohamed *et al*. (2019) reported a significantly higher percentage of participants (31.2%) consumed under-cooked meat as compared to the 10.68% found in the current study. This is concerning, since the main route of transmission of TB from animal to humans is via the ingestion of raw meat, unpasteurized milk and contaminated droplets from an infected animal. This agrees with studies that identified the consumption of raw meat and unpasteurized milk products as a factor that promotes infection between cattle and humans (Sa'idu *et al*., 2014). Moreover, there is a growing habit of consumption of raw meat and unpasteurized milk, especially in Africa (van Helden & Michel, 2019; Sichewo *et al*., 2020). Unhygienic food habits are among the factors that expose the respondents to the transmission of zoonotic diseases (Swai *et al*., 2010).

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Over 40% of abattoir workers in the current study slaughter animals at home, which has been identified as an unsafe slaughtering practice, and is one of the factors that predisposes abattoir workers to the transmission of zoonotic diseases. This practice has been reported to be among the factors that were reported in some African countries that enhance the transmission of zoonotic TB (Swai *et al*., 2010). This study further revealed that the age group 36-50 years had the highest frequency of respondents that slaughtered livestock at home. Taking care and slaughtering of animals at home are some of the factors that predispose abattoir workers to infection with the pathogen. This is due to close contact with live animals which may results in transmission of the disease by inhalation (Melaku *et al*., 2013).

In this study, three variables (such as if they have ever been sick witk with TB, awareness on zoonotic nature of TB) were detected to have statistically significant differences (meaning the values of $p<0.05$ were regarded as statistically significant) in the frequencies among the abattoir workers. Although less than half of the workers were aware of the zoonotic nature of TB, there was a statistically significant difference (meaning values of $p<0.05$ were regarded as statistically significant) ($P=0.030$) in the frequency of workers who were knowledgeable that humans can transmit TB to animals, which was detected at the highest frequency in the 26-35 age group (39.02%). This means that individuals in this age group were more knowledgeable as compared to the age group 18-25 who were least knowledgeable on the zoonotic potential of TB. These findings agree with previous studies that revealed that an increase in age contributed to increasing knowledge as the higher age groups have more working experience in comparison to those below that age group (Ismaila *et al*., 2015).

It is also relevant to mention that the differences in the frequency of abattoir workers who had TB were statistically significant ($P=0.009$), (values of $p<0.05$ were regarded as statistically significant, with the highest frequency) (57.14%) detected in those aged 36 – 50 years old. A total of 18.2% of the workers responded that they had a family member that was previously

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diagnosed with TB. It is expected that the respondents who know the signs and symptoms of TB and its transmission are more likely to practise preventive measures that will reduce exposure to TB compared to those with low knowledge of the disease (Adebanjo, 2011). Other studies have demonstrated that knowledge of the transmission of TB leads to lower prevalences of TB being detected in communities (Sreeramareddy & Arokiasamy, 2013; Tschopp *et al.*, 2015).

Although most of the symptoms associated with TB were not exhibited or experienced at a statistically significant level among the abattoir workers, the frequency of night sweats was the only symptom observed to be statistically significantly (P=0.021) different among the abattoir workers. Furthermore, the symptom was detected at the highest frequency (57.14%) of the workers with the knowledge of TB.

Limitations to the study were that the questionnaire did not capture information to address the employee perception of tuberculosis.

5.8 Conclusion

The study identified factors associated with the transmission of bovine tuberculosis TB among red meat abattoir workers in Gauteng province of South Africa by assessing and analyzing KAP regarding TB and their potential exposure to the pathogen. Since abattoir workers are in a high-disease risk occupation, efforts should be made by health authorities together with the abattoir management to train abattoir workers regularly, not only on bTB transmission but also on other zoonotic diseases such as brucellosis and leptospirosis. There should also be increased awareness programmes, especially on TB, among abattoir workers regarding disease prevention, transmission, and treatment relevant to the settings where they work. This may lead

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to a reduction of risks posed to abattoir workers, in turn, their families and communities as well.

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5.9 References

Adebanjo, O.D., 2011. *Knowledge, attitudes, and practices of healthcare workers about prevention and control of multidrug-resistant tuberculosis at Botsabelo Hospital Maseru, Lesotho* (Doctoral dissertation, University of Limpopo (Medunsa Campus).

Adesokan, H.K., Akinseye, V.O. and Sulaimon, M.A., 2018. Knowledge and practices about zoonotic tuberculosis prevention and associated determinants amongst livestock workers in Nigeria; 2015. *PLoS One*, *13*(6), p.e0198810.

Ayele, W.Y., Neill, S.D., Zinsstag, J., Weiss, M.G. and Pavlik, I., 2004. Bovine tuberculosis: an old disease but a new threat to Africa. *The International Journal of Tuberculosis and Lung Disease*, *8*(8), pp.924-937.

Cosivi, O., Grange, J.M., Daborn, C.J., Raviglione, M.C., Fujikura, T., Cousins, D., Robinson, R.A., Huchzermeyer, H.F., de Kantor, I. and Meslin, F.X., 1998. Zoonotic tuberculosis due to *Mycobacterium bovis* in developing countries. *Emerging Infectious Diseases*, *4*(1), p.59.

Datiko, D.G., Habte, D., Jerene, D. and Suarez, P., 2019. Knowledge, attitudes, and practices related to TB among the general population of Ethiopia: Findings from a national cross-sectional survey. *PloS One*, *14*(10), p.e0224196.

De la Rua-Domenech, R., 2006. Human *Mycobacterium bovis* infection in the United Kingdom: incidence, risks, control measures and review of the zoonotic aspects of bovine tuberculosis. *Tuberculosis*, *86*(2), pp.77-109.

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Ismaila, U.G., Rahman, H.A. and Saliluddin, S.M., 2015. Knowledge on bovine tuberculosis among abattoir workers in Gusau, Zamfara State, Nigeria. *International Journal of Public Health and Clinical Sciences*, *2*(3), pp.45-58.

Kachalla, M.G., Kwaghe, A.V., Bello, M. and Nguku, P., 2015. Knowledge, Attitude and Practice of Workers on Bovine Tuberculosis (bTB) in Two Abuja Abattoirs, Federal Capital Territory, Nigeria. *International Journal of Life Sciences, 5*(2), pp.124-128.

LoBue, P.A., Enarson, D.A. and Thoen, C.O., 2010. Tuberculosis in humans and animals: an overview [Serialised article. Tuberculosis: a re-emerging disease in animals and humans. Number 1 in the series]. *The International Journal of Tuberculosis and Lung Disease*, *14*(9), pp.1075-1078.

Marange, R., Morar-Leather, D. and Fasina, F.O., 2020. Survey of the knowledge, attitude, and perceptions on bovine tuberculosis in Mnisi community, Mpumalanga, South Africa. *Onderstepoort Journal of Veterinary Research*, *87*(1), pp.1-4.

Melaku, S., Sharma, H.R. and Alemie, G.A., 2013. Pastoralist community's perception of tuberculosis: A quantitative study from Shinille area of Ethiopia. *Tuberculosis Research and Treatment*, *2013*, pp.1-8.

Michel, A.L., Geoghegan, C., Hlokwe, T., Raseleka, K., Getz, W.M. and Marcotty, T., 2015. Longevity of *Mycobacterium bovis* in raw and traditional souring milk as a function of storage temperature and dose. *PloS One*, *10*(6), p.e0129926.

Miller, M., White, P.C. and Bengis, R.G., 2015. Tuberculosis in South African wildlife: why is it important? *Sun Media: Stellenbosch, South Africa*.

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Ogundeji, E., Onyemelukwe, N., Nwuko, A., Onuoha, M., Ogundeji, A., Osaretin, J., Eze, K., Olofu, J., Jik, A., Kemza, S. and Okafor, N.C., 2015. Molecular detection of *Mycobacterium bovis* in cattle milk in Enugu State, Nigeria. *Journal Natural Science Research*, *5*, pp.42-47.

Olea-Popelka, F., Muwonge, A., Perera, A., Dean, A.S., Mumford, E., Erlacher-Vindel, E., Forcella, S., Silk, B.J., Ditiu, L., El Idrissi, A. and Raviglione, M., 2017. Zoonotic tuberculosis in human beings caused by *Mycobacterium bovis*—a call for action. *The Lancet Infectious Diseases*, *17*(1), pp.e21-e25.

Raviglione, M.C. and Korobitsyn, A.A., 2016. End TB-the new era WHO strategy in the SDG era and the contributions from the Russian Federation. *Tuberculosis and Lung Diseases*, *94*(11), pp.7-15.

Rogall, T., Wolters, J., Flohr, T. and Bottger, E.C., 1990. Towards a phylogeny and definition of species at the molecular level within the genus *Mycobacterium*. *International Journal of Systematic and Evolutionary Microbiology*, *40*(4), pp.323-330.

Sa'idu, A.S., Okolocha, E.C., Dzikwi, A.A., Gamawa, A.A., Ibrahim, S., Kwaga, J.K.P., Usman, A. and Maigari, S.A., 2015. Public health implications and risk factors assessment of *Mycobacterium bovis* infections among abattoir personnel in Bauchi state, Nigeria. *Journal of Veterinary Medicine*, *2015*, pp.1-5.

Sichewo, P.R., Vander Kelen, C., Thys, S. and Michel, A.L., 2020. Risk practices for bovine tuberculosis transmission to cattle and livestock farming communities living at wildlife-livestock-human interface in northern KwaZulu Natal, South Africa. *PLoS Neglected Tropical Diseases*, *14*(3), p.e0007618.

Sreeramareddy, C.T., Harsha Kumar, H.N. and Arokiasamy, J.T., 2013. Prevalence of self-reported tuberculosis, knowledge about tuberculosis transmission and its determinants among

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adults in India: results from a nation-wide cross-sectional household survey. *BMC infectious diseases*, *13*(1), pp.1-9.

Swai, E.S., Schoonman, L. and Daborn, C., 2010. Knowledge and attitude towards zoonoses among animal health workers and livestock keepers in Arusha and Tanga, Tanzania. *Tanzania Journal of Health Research*, *12*(4), pp.272-277.

Tschopp, R., Bekele, S., Moti, T., Young, D. and Aseffa, A., 2015. Brucellosis and bovine tuberculosis prevalence in livestock from pastoralist communities adjacent to Awash National Park, Ethiopia. *Preventive Veterinary Medicine*, *120*(2), pp.187-194.

van Helden, P.D. and Michel, A., 2019. Bovine TB Zoonosis in Africa. In *Tuberculosis in animals: an African perspective* (pp. 31-40). Springer, Cham.

Vayr, F., Martin-Blondel, G., Savall, F., Soulat, J.M., Deffontaines, G. and Herin, F., 2018. Occupational exposure to human *Mycobacterium bovis* infection: A systematic review. *PLoS Neglected Tropical Diseases*, *12*(1), p.e0006208.

World Health Organisation, 2013. *Global Tuberculosis Report: epidermiology, prevention control and economics: WHO report 2013.* World Health Organisation.

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Chapter 6. Application of the gamma-interferon assay to determine the prevalence of bovine tuberculosis in slaughter livestock at abattoirs in Gauteng, South Africa

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ORIGINAL ARTICLE

WILEY

Application of the gamma-interferon assay to determine the prevalence of bovine tuberculosis in slaughter livestock at abattoirs in Gauteng, South Africa

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6.1. Summary

Background: Bovine tuberculosis (bTB) is a zoonotic disease with great economic impact estimated at billions of dollars annually worldwide. Meat inspection represents a long-standing form of disease surveillance that serves both food safety and animal health. The objective of this study was to determine the prevalence of bTB in livestock at abattoirs using a cell-mediated immune (CMI) assay, the interferon-gamma (IFN-γ) assay. This cross-sectional study was conducted at selected abattoirs (low-throughput, high-throughput and rural/informal) in Gauteng province, where animals were also subjected to routine meat inspection.

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Results: A total of 410 fresh blood samples were collected from slaughter livestock (369 cattle and 41 sheep) from 15 abattoirs and analysed using a Bovigam® test kit with bovine, avian and Fortuitum purified protein derivatives (PPD) as blood stimulating antigens. The estimated prevalence of bTB in cattle was 4.4% (95% Confidence Interval: 2.4-7.3%). The prevalence of bTB in cattle varied between abattoirs (p=0.005), ranging from 0 to 23%; however, there were no significant differences among genders, breeds, municipality, districts, and origins of animals (feedlot, auction, or farm) or throughput of abattoirs. The prevalence of avian reactors was 6.0% (95% Confidence Interval: 3.6-9.2%) in cattle, varying between abattoirs (P=0.004) and ranging from 0 to 20.7%. None of the sheep with valid test results were positive for bTB and none were avian reactors (95% Confidence Interval: 0-15%).

Conclusion: The detection of bTB reactor cattle in our study clearly shows the limitation of disease surveillance using a meat inspection approach, as all the 410 slaughter animals sampled had passed visual abattoir inspection and been classified as bTB-free. Our findings, therefore, emphasize the risk of zoonotic transmission of bTB to abattoir workers and potential food safety hazards to consumers. Furthermore, our study highlights the potential for the use of the IFN-γ assay to reduce this risk.

Keywords: Abattoirs; bovine tuberculosis, gamma interferon assay; cattle; zoonosis

6.2. Impact of the study

- The main aim of this study was to determine the prevalence of bovine tuberculosis (bTB) in slaughter livestock at abattoirs in Gauteng province of South Africa using the modified interferon-gamma (IFN-γ) assay.
- Our study highlighted the inadequacy of meat inspection alone to detect bTB in cattle slaughtered for human consumption; hence imperative to apply additional methods,

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such as the IFN-γ assay to establish the bTB infection status in slaughter cattle at abattoirs.

- This approach will likely reduce the risk of TB posed to abattoir workers and consumers of meat from infected cattle.

Introduction

Mycobacterial species that are responsible for tuberculosis infections in both humans and animals belong to the *Mycobacterium tuberculosis* complex (MTBC). The complex comprises organisms such as *Mycobacterium tuberculosis*, *Mycobacterium bovis* (Karlson & Lessel, 1970), *M. bovis* Bacillus Calmette–Guerin (BCG), *Mycobacterium africanum*, *Mycobacterium microti*, *Mycobacterium canettii*, *Mycobacterium pinipeddii* (Cousins, Bastida, Cataldi, Quse, Redrobe, Dow, Duignan, Murray, Dupont, Ahmed, Collins, Butler, Dawson, Rodriguez, Loureiro, Romano, Alito, Zumarraga, & Bernardelli, 2003) and M*ycobacterium caprae* (Aranaz, Liebana, Gomez-Mampaso, Galan, Cousins, Ortega, Blazquez, Baquero, Mateos, Suarez, & Dominduez, 1999). Additionally, species such as *Mycobacterium orygis* (Van Ingen, Rahim, Mulder, Boerre, Simeone, Brosch, & van Solingen, 2012), *Mycobacterium mungi* (Alexander, Laver, Michel, Williams, van Helden, Warren, & Gey van Pittius, 2010) and *Mycobacterium suricattae* (Parsons, Drewe, Gey van Pittius, Warren, & Helden, 2013) have been identified recently. The MTBC complex has high levels of genetic similarity, up to 99.9%, amongst its members (Rogal, Wolters, Flohr, & Botter, 1990) and is responsible for infecting different host species. Bovine tuberculosis (bTB), which is mainly caused by *M. bovis,* is considered one of the largest veterinary health problems and a public health concern (Michel, Muller, & Helden, 2010). Organisations such as World Organisation for Animal Health (OIE), World Health Organisation (WHO), and Food and Agriculture Organisation (FAO) have classified bTB as a neglected zoonotic disease, especially in developing countries.

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Approximately 50 million cattle are infected annually worldwide, resulting in economic losses of approximately $3 billion (Waters, Palmer, Buddle, & Vordermeier, 2012). Due to the scourge of the disease, WHO declared a global emergency in 1993 (Grange & Zumla, 2002).

Bovine TB has a wide host range consisting of wild animals, domestic animals and humans and the hosts are categorised into two groups, i.e. the maintenance and the spill-over or dead-end hosts (Ayele, Neill, Zinsstag, Weiss, & Pavlik, 2004). In South Africa, bTB is endemic and *M. bovis* infection has been reported in cattle, pigs and 21 different wildlife species (Hlokwe, van Helden, & Michel, 2014; Hlokwe, Michel, Mitchel, Gcebe, & Reininghaus, 2019; Michel, 2008). A recent study by Hlokwe and colleagues (2014) demonstrated that bTB had not only increased in spatial distribution in South Africa but that the number of wildlife that can be infected by *M .bovis* has also increased in comparison to a decade ago (Hlokwe, Michel, Mitchel, Gcebe, & Reininghaus, 2019). The prevalence of bTB in commercial cattle herds in South Africa was reduced to 0.4% in 1995 due to implementation of the test and slaughter programme which was introduced in 1969 (Michel, 2008). In recent years, sporadic outbreaks in different regions have been reported (Rodwell, Kriek, Bengis, Whyte, Viljoen, de Vos, & Boyce, 2001; Hlokwe, van Helden, & Michel, 2014; Sichewo, Etter, & Michel, 2019).

Early and accurate diagnosis of bTB is important to limit the spread of the disease because of its chronic nature (Churbanov & Milligan, 2012). Globally, abattoirs are used for passive and active surveillance of diseases of both economic and public health significance. Information generated from abattoir surveillance could provide an early warning system for impending epidemics or failures of intervention programmes (Alton, Pearl, Bateman, McNab, & Berke, 2015).

The Bovigam® test (Prionics AG, Lelystad, The Netherlands) is a blood-based cell-mediated immune assay that has been validated, included in the World Organisation for Animal Health

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(OIE) register as a diagnostic kit for bTB and is commonly applied as an ancillary test for the ante-mortem diagnosis of bTB in cattle, goats, sheep (Munoz-Mendoza, Romero, del Cerro, Gortazar, Garcia-Marin, Menendez, Mourelo, de Juan, Saez, Delahay, & Balseiro, 2016) and buffaloes (Michel, Cooper, Jooste, de Klerk, & Jolles, 2011). The test is used to detect latent bTB by measuring the amount of IFN-γ released by white blood cells upon infection and may be modified by inclusion of Fortuitum purified protein derivative (PPD) as an additional antigen to increase its specificity (unpublished data). The current study was conducted at selected abattoirs in the Gauteng province, South Africa and the main objective was to determine the prevalence of livestock bTB in slaughter livestock in these abattoirs using the modified IFN-γ assay. The results should indicate the status of bTB in livestock farms from where the animals originated.

6.1. Materials and Methods

6.1.1. Type of study, sample size determination and animal species

This was a cross-sectional study conducted at selected abattoirs (2 low throughput and 13 high throughput) in Gauteng province of South Africa. The required sample size of 410 was determined using a formula (Thrusfield, Christley, Brown, Diggle, French, Howe, Kelly, O'Connor, Sargeant, & Wood, 2013) to estimate a prevalence of 1% with a 1% precision. At each selected abattoir, slaughter cattle and/or sheep were sampled on a single day using a systematic random sampling method. A total of 410 samples were collected comprising 369 adult cattle (Bonsmara, n=277; Jersey, n= 39; Nguni, n= 51; Brahman, n= 1; Holstein, =1) and 41 sheep (Dorper) of any gender for serological testing for livestock bTB using the IFN-γ assay.

6.1.2. Pre and post-slaughter inspection

The professional meat inspectors assigned to each abattoir conducted pre- and post-slaughter inspection according to a standard procedure, which included the detection of clinical TB lesions. Pre-slaughter inspection was conducted by checking for abnormalities in respiration,

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behaviour etc. of the animal that was being inspected. A post-slaughter inspection was performed as follows: the internal and external surfaces of the carcass were examined for the detection of any lesions. For the head inspection, lymph nodes such as retropharyngeal lymph node, parotid, and submaxillary were incised and examined. In cattle, the oesophagus was separated and examined. For inspection of the viscera in sheep, the bronchi were opened and in cattle, the larynx, trachea, and bronchi were opened and examined. The heart was incised from the base to the apex and examined. The hepatic system and the liver were also excised and examined. Other organs such as the stomach, spleen including its lymph nodes, and the gastro intestinal tract were also examined. Any part of the carcass that showed abnormalities was condemned. Information regarding animal species, gender, breed, district, and municipality, origin of animals, abattoir name and throughput was recorded in a spreadsheet.

6.1.3. Blood collection

During the slaughter of animals, blood samples were collected into sterile heparinised tubes, transported to the laboratory at room temperature, and processed within 8 h of collection. All samples were processed according to established standard laboratory protocols used at the Tuberculosis laboratory of the Agricultural Research Council-Onderstepoort Veterinary Research (ARC-OVR), Onderstepoort, South Africa.

6.1.4. Stimulation of whole blood samples and detection of gamma interferon

In the first phase of the test, fresh blood samples were stimulated with purified protein derivatives (PPD). For each animal, blood in the heparin tube was carefully mixed and 1.5 ml aliquoted into 5 individual wells of a 24-well plate. Blood samples were sensitized with 30 µl bovine PPD (600 IU/ml); 60 µl avian PPD (1000 IU/ml) (Prionics AG, Lelystad, The Netherlands); 25 µl PPD-Fortuitum (0.5 mg/ml) in individual wells. As an internal positive control, 11 µl Pokeweed mitogen (5 µg/ml) was aliquoted into the next well of the plate. Unstimulated whole blood from each animal served as negative controls for the assay. The

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tuberculin and blood were carefully mixed by gentle hand agitation and incubated at 37°C for 20-24 h. After incubation, the samples were centrifuged at 3000 rpm for 10 minutes and 150 µl of the plasma was transferred into appropriately identified tubes with corresponding labels. Plasma samples were stored at -20°C until tested. The plasma samples were assayed for the presence of IFN-γ using a commercially purchased Bovigam® 1G-test kit (Prionics AG, Lelystad, The Netherlands), following the manufacturer's instructions. The production of IFN-γ by the lymphocytes was detected using a monoclonal antibody-based sandwich enzyme immunoassay (EIA). Optical densities were measured on a BioTek ELx800 Plate reader (BioTek Instruments Inc., Winooski, Vermont, USA) at 450 nm. Results were interpreted as previously described (Michel, Cooper, Jooste, de Klerk, & Jolles, 2011).

6.1.5. Statistical analysis

Univariate associations of breed, gender, district, municipality, abattoir, origin of animals, abattoir type and animal species with the prevalence of bTB, of avian reactors, and of overall *Mycobacterium* spp. exposure, were assessed using cross-tabulation and the Fisher's exact test. Where possible, variables significant in the univariate analysis were included in multivariable logistic or exact logistic regression models to adjust for confounding. Data were analysed using Stata 15 (StataCorp, College Station, TX, USA); and $p<0.05$ was regarded as statistically significant.

6.2. Results

Of the 369 cattle sampled, valid IFN-γ results (i.e. test samples passed quality control checks) were obtained in 318 (86.2%) of the cattle. The estimated prevalence of cattle positive for bTB was 4.4% (95% Confidence Interval: 2.4-7.3%) (Table 6.1). Of the eight variables analysed, seven (animal species, gender, breed, district, municipality, origin of animals, and abattoir throughput) were not associated with the estimated prevalence of bTB. However, prevalence varied significantly between abattoirs ($p=0.005$), ranging between 0% and 23.1% (Table 6.1).

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The estimated prevalence of avian reactors was 5.9% (95% Confidence Interval: 3.6-9.2%) (Table 6.2), also varying significantly between abattoirs (p=0.004), ranging from 0% to 20.7%. The prevalence of avian reactors in cattle was not significantly different to that of bTB. The estimated prevalence of cattle tested reacting to *Mycobacterium* spp. (combined bTB and avian reactors) was 10% (95% Confidence Interval: 7.0-14%) (Table 6.3). In the univariate analysis, prevalence varied by gender of animal (3.0% in females and 11.9% in males) and by breed (5.4% in Jersey, 13% in Bonsmara, 0% in other breeds), but these differences were not significant after adjusting for confounding using exact logistic regression. Of the 41 sheep sampled, valid IFN-γ results were obtained in 22 (54%) of the animals and none were positive for bTB nor were there any avian reactors (95% Confidence Interval: 0-15%) (Table 6.1-6.3). No nodular bTB lesions were detected in any of the slaughtered livestock during post mortem examination.

6.3. Discussion

Detection of bTB and avian reactors in our study is an indication that IFN-γ was released from T-lymphocytes of cattle infected with a member of the MTBC or *Mycobacterium avium* species respectively. While MTBC species are the causative agents of bTB, *Mycobacterium avium* species are non-tuberculous mycobacteria that do not cause bTB in cattle. The IFN-γ assay has long been used for the detection of bTB in cattle (Gormley, Doyle, Fitzsimons, McGill, & Collins, 2006) and buffalo (Michel, Cooper, Jooste, de Klerk, & Jolles, 2011). One of its advantages is that it can detect early stages of infection as compared to post-mortem examination of lesions that are a result of late stages of infection.

The IFN-γ assay is recognised by authorities such as the European Union and in countries such as New Zealand as an approved test for the diagnosis of bTB (Ozturk, Pehlivanoglu, Turutoglu, Kale, Guldali, & Tok, 2010) and it has been used in many countries for surveillance and in bTB eradication control programs as an ancillary test or a confirmatory test. A study conducted

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by Ozturk and colleagues (2010) in Turkey revealed comparable sensitivity and specificity between the intradermal test and the IFN-γ assay. The study showed that the IFN-γ assay had a sensitivity of 90% and specificity of 97% and they recommended that it could be used as an alternative to the intradermal tuberculin test (Ozturk, Pehlivanoglu, Turutoglu, Kale, Guldali, & Tok, 2010). In South Africa, the IFN-γ assay was modified (by the inclusion of Fortuitum PPD as an additional antigen) to counter the observed false-positive test results caused by cross-reaction with environmental mycobacteria in cattle, providing a high specificity of over 99% and sensitivity of 86% in cattle (unpublished data). This modified Bovigam® test has since been applied to both cattle and buffaloes in South Africa (Hlokwe, De Klerk-Lorist & Michel, 2016; Sichewo, Etter, & Michel, 2019).

In the current study, we applied the IFN-γ assay in slaughter livestock and estimated the prevalence of bTB in cattle to be 4.4%. Variables such as species, breed, abattoir throughput and source of animals were not associated with the prevalence, although it varied significantly between abattoirs, suggesting geographic variation in the prevalence of bTB in and around Gauteng.

No MTBC infection in sheep was detected, although our sample size was small; moreover, a high percentage (46%) of invalid test results were obtained in sheep. Bovine tuberculosis in sheep has never been reported in South Africa and the true disease status is currently unknown. Additionally, the sensitivity and specificity of the IFN-γ assay has also not been evaluated in sheep. Although the infection in sheep is generally uncommon worldwide, in countries such as Spain, several outbreaks of the disease epidemiologically linked to cattle have been reported (Munoz-Mendoza, Romero, del Cerro, Gortazar, Garcia-Marin, Menendez, Mourelo, de Juan, Saez, Delahay, & Balseiro, 2016). A recent report from Ethiopia (Gelalcha, Zewude, & Ameni, 2019) indicated that sheep serve as spill-over hosts as they become infected only if there is a source of infection and sheep-to-sheep transmission of *M. bovis* is highly unlikely.

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Meat inspection is a long-standing form of disease surveillance for both food safety and animal health. For diseases that produce slow progressive but evident lesions, such as bTB, slaughterhouse inspection is considered an effective surveillance tool (Gormley, Corner, Costello, & Rodriguez-Campos, 2014). The detection of positive bTB reactors in our study has, however, clearly shown the limitations of this method of disease surveillance, as the carcasses of all the 410 slaughter animals sampled had passed visual meat inspection and been classified as TB-free. It is, however, possible that visible lesions had not yet formed, as they represent late stage of infection. It is known that abattoir workers are faced with a high risk of exposure to bTB since they might be unknowingly inspecting an infected carcass in closed spaces, which may lead to direct inhalation of contaminated droplets (Vayr *et al.*, 2018). Additionally, exposure might occur as a result of occupational injuries since they are working with knives (de la Rua-Domenech, 2005; Vayr, Martin-Blondel, Savall, Soulat, Deffontaines, & Herin, 2018). Hence, the potential zoonotic risk of transmission to abattoir workers as well as a food safety hazard to consumers cannot be ignored. The risk might, however, be lessened in meat consumers provided that the meat is well cooked.

The IFN-γ assay is a rapid test, with results available within 48 hours following blood collection and it is cost-effective. Although it may not be feasible to test every animal destined for slaughter, a structured screening mechanism may be developed for testing animals originating from the same farm to determine their disease status. Studies have demonstrated that the use of the IFN-γ assay in combination with other TB diagnostics tests leads to more accurate screening for bTB in cattle (Ahir, Filia, Mahajan, Leishangthem, Rai, & Singh, 2016; Neeraja, Veeregowda, Sobha Rani, Rathnamma, Narayanaswamy, Venkatesha, Leena, Apsana, Somshekhar, Saminathan, Dhama, & Chakraborty, 2014).

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6.4. Conclusion

This study demonstrated the presence of bTB in animals classified as TB-free by routine meat inspection in abattoirs in Gauteng. Our study further highlighted the inadequacy of meat inspection alone to detect bTB in cattle slaughtered for human consumption. It is therefore imperative to apply additional methods, such as the IFN-γ assay, to establish the bTB infection status in slaughter cattle at abattoirs. This approach will likely reduce the risk of TB posed to abattoir workers and consumers of meat from infected cattle.

6.5. Conflicting interest

The authors declare no conflict of interest.

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6.6. References

Ahir, P., Filia, G., Mahajan, V., Leishangthem, G.D., Rai, T.S. & Singh, A. (2016). Diagnosis of bovine tuberculosis in lactating cattle and buffaloes by comparative intradermal tuberculin test and

bovine gamma interferon immunoassay. *Journal of Animal Research*, 6, 1069-1072. doi: 10.5958/2277-940X.2016.00157.1.

Alexander, K. A., Laver, P. N., Michel, A. L., Williams, M., van Helden, P. D., Warren, R. M. & Gey van Pittius, N. C. (2010). Novel Mycobacterium tuberculosis complex pathogen, M. Mungi. *Emerging Infectious Diseases*, 8, 598-601. doi: 10.3201/eid1608.100314.

Alton, G.D., Pearl, D.L., Bateman, K.G., McNab, W.B., Berke, O. (2015). Suitability of sentinel abattoirs for syndromic surveillance using provincially inspected bovine abattoir condemnation data. *BMC Veterinary Research*, doi: 10.1186/s12917-015-0349-1.

Aranaz, A., Liebana, E., Gomez-Mampaso, E., Galan, J. C., Cousins, D., Ortega, A., Blazquez, J., Baquero, F., Mateos, A., Suarez, G. & Dominguez, L. (1999). *Mycobacterium tuberculosis* subspecies, *caprae* subspecies: a taxonomic study of a new member of the *Mycobacterium tuberculosis* complex isolated from goats in Spain. *International Journal of Systematic Bacteriology*, 49, 1263-1273. doi: 10.1099/00207713-49-3-1263.

Ayele, W. Y., Neill, S. D., Zinsstag, J., Weiss, M. G., Pavlik, I. (2004). Bovine tuberculosis: an old disease but a new threat to Africa. *International Journal of Tuberculosis and Lung Disease*, 8, 924-937.

Churbanov A., B. & Milligan. (2012). Accurate diagnosis of bovine tuberculosis based on high throughput sequencing. *Plos One*, 7(11), 1-9. doi: 10.1371/journal.pone.0050147.

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Cousins, D. V., Bastida, R., Cataldi, A., Quse, V., Redrobe, S., Dow, S., Duignan, P., Murray, A., Dupont, C., Ahmed, N., Collins, D. M., Butler, W. R., Dawson, D., Rodriguez, D., Loureiro, J., Romano, M. I., Alito, A., Zumarraga, M. & Bernardelli, A. (2003). Tuberculosis in seals caused by a novel member of the *Mycobacterium tuberculosis* complex: *Mycobacterium pinnipedii* sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, 53, 1305-1314. doi: 10.1099/ijs.0.02401-0.

de la Rua-Domenech, R. (2005). Human *Mycobacterium bovis* infection in the United Kingdom: Incidence, risks, control measures and review of the zoonotic aspect of bovine tuberculosis. *Elsevier*, 86, 77-109. doi: 10.1016/j.tube.2005.05.002

Gelalcha, B. D., Zewude, A. & Ameni, G. (2019). Tuberculosis caused by *Mycobacterium bovis* in a sheep flock colocated with a tuberculosis dairy cattle herd in Central Ethiopia. *Journal of Veterinary Medicine*, doi: 10.1155/2019/8315137.

Gormley, E., Doyle, M. B., Fitzsimons, T., McGill, K. & Collins, J. D. (2006) Diagnosis of *Mycobacterium bovis* infection in cattle by use of the gamma-interferon (Bovigam®) assay. *Veterinary Microbiology*, 112, 171-179. doi: 10.1016/j.vetmic.2005.11.029.

Gormley, E., Corner, L. A. L., Costello, E. & Rodriguez-Campos, S. (2014). Bacteriological diagnosis and molecular strain typing of *Mycobacterium bovis* and *Mycobacterium caprae*. *Research in Veterinary Science*, 97, S30-S43. doi: 10.1016/j.rvsc.2014.04.010.

Grange, J. M. & Zumla, A. (2002). The global emergency of tuberculosis: What is the cause? *The Journal of the Royal Society for the Promotion of Health*, 122, 78-81. doi:10.1177/146642400212200206.

Hlokwe, T. M., van Helden, P. & Michel, A. (2014). Evidence of increasing intra and inter-species transmission of *Mycobacterium bovis* in South Africa: Are we losing the battle? *Preventive Veterinary Medicine*, 115, 10-17. doi: 10.1016/j.prevetmed.2014.03.011.

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Hlokwe, T., De Klerk-Lorist, L-M., Michel, A. (2016). Wildlife on the move: A hidden tuberculosis threat to conservation areas and game farms through translocation of untested animals. *Journal of Wildlife Diseases*, 52 (4): doi: 10.7589/2015-10-281.

Hlokwe, T. M., Michel, A. L., Mitchel E., Gcebe, N. & Reininghaus, B. (2019). First detection of *Mycobacterium bovis* in Giraffe (*Giraffa camelopardalis*) in Greater Kruger National Park Complex: Role and Implications. *Transboundary and Emerging Disease*, 66, 2264-2270. doi: 10.1111/tbed.13275.

Karlson, A. G. & Lessel, E. F. (1970). *Mycobacterium bovis*. *International Journal of Systematic Bacteriology*, 20, 273-282. doi:10.1099/00207713-20-3-273.

Michel, A. (2008). *Mycobacterium fortuitum* infection interference with *Mycobacterium bovis* diagnostics: natural infection cases and a pilot experimental infection. *Journal of Veterinary Diagnostic Investigation*, 20, 501-503. doi: 10.1177/104063870802000415.

Michel, A. L., Muller, B. & Helden, P. D. (2010). *Mycobacterium bovis* at the animal-human interface: A problem or not? *Veterinary Microbiology,* 140 (3), 371-381. doi: 10.1016/j.vetmic.2009.08.029.

Michel, A. L., Cooper, D., Jooste, J., de Klerk L.-M. & Jolles, A. (2011). Approaches towards optimising the gamma interferon assay for the diagnosing *Mycobacterium bovis* in African buffalo (*Syncerus caffer*). *Preventive Veterinary Medicine,* 98, 142-151. doi: 10.1016/j.prevetmed.2010.10.016.

Munoz-Mendoza, M., Romero, B., del Cerro, A., Gortazar, C., Garcia-Marin, J.F., Menendez, S., Mourelo, J., de Juan, L., Saez, J.L., Delahay, R.J., & Balseiro, A. (2016). Sheep as potential source of bovine TB: Epidemiology, pathology and evaluation of diagnostic techniques. *Transboundary and Emerging Diseases*, 63, 635-646. doi: 10.1111/tbed.12325.

© University of Pretoria

Neeraja, D., Veeregowda, B. M., Sobha Rani, M., Rathnamma, D., Narayanaswamy, H. D., Venkatesha, M. D., Leena, G., Apsana, R., Somshekhar, S. H., Saminathan, M., Dhama K., & Chakraborty, S. (2014). Identification of *Mycobacterium tuberculosis* Complex by Culture and Duplex Polymerase Chain Reaction in Bovines. *Asian Journal Animal and Veterinary Advances*, 8, 506-512. doi: 10.3923/ajava.2014.506.512.

Ozturk, D., Pehlivanoglu, F., Turutoglu, H., Kale, M., Guldali, Y. & Tok, A. A. (2010). In Vitro diagnosis of bovine tuberculosis by γ-Interferon assay. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 16, 875-878. doi: 10.9775/kvfd.2015.13293.

Parsons, S. D. C., Drewe, J. A., Gey van Pittius, N. C., Warren, R. M. & Helden, P. D. (2013). Novel cause of Tuberculosis in Meerkats, South Africa. *Emerging Infectious Diseases*, 12, 2004-2007. doi: 10.3201/eid1912.130268.

Rodwell, T. C., Kriek, N. P., Bengis, R. G., Whyte, I. J., Viljoen P. C., de Vos V. & Boyce W. M. (2001). Prevalence of bovine tuberculosis in African buffalo at Kruger National Park. National Park. *Journal of Wildlife Diseases*, 37, 258-264. doi: 10.7589/0090-3558-37.2.258.

Rogal, T., Wolters, J., Flohr, T. & Botter, E. (1990). Towards a phylogeny of species at the molecular level within the Genus Mycobacterium. *International Journal of Systematic Bacteriology*, 40, 323-330. doi: 0020-7713/90/040323-08$02.00/0.

Sichewo, P. R., Etter, E. M. C. & Michel, A. L. (2019). Prevalence of *Mycobacterium-bovis* infection in traditionally managed cattle at the wildlife-livestock interface in South Africa in the absence of control measures. *Veterinary Research Communications*, 43, 155-164. doi: 10.1007/s11259-019-09756-w.

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Thrusfield M. V, Christley, R., Brown, H., Diggle, P. J., French, N., Howe, K., Kelly, L., O'Connor, A., Sargeant, J. & Wood, H. (2013). *Veterinary Epidemiology* (4th ed.)., Oxford, Wiley Blackwell.

Van Ingen, J., Rahim, Z., Mulder, A., Boerre, M. J., Simeone, R., Brosch, R. & van Solingen, D. (2012). Characterisation of *Mycobacterium orygis* as *M. tuberculosis* complex subspecies. *Emerging Infectious Diseases*, 18, 653-655. doi: 10.3201/eid1804.110888.

Vayr, F., Martin-Blondel, G., Savall, F., Soulat, J., Deffontaines, G. & Herin, F. (2018). Occupational exposure to human *Mycobacterium bovis* infection: A systematic review. *PLOS Neglected Tropical Diseases*, 12(1), 1-14. doi: 10.1371/journal.pntd.000628.

Waters, W. R., Palmer, M. V., Buddle, B. M. & Vordermeier. (2012). Bovine tuberculosis vaccine research: Historical perspective and recent advances. *Vaccine*, 30, 2611-2622. doi: 10.1016/j.vaccine.2012.02.018

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Table 6.1 Overall estimated prevalence of bovine tuberculosis (bTB) in cattle in Gauteng abattoirs

Variable	Level	n	Prevalence (%)	95% CI	p-value
Species	Bovine	318	4.4	2.4-7.3	0.612
	Ovine	22	0	0-15.4	
Sex	Male	252	5.2	2.8-8.7	0.315
	Female	66	1.5	0.04-8.2	
Breed	Bonsmara	231	5.6	3.0-9.4	0.27
	Nguni	48	0	0-7.4	
	Jersey	37	2.7	0.07-14.2	
	Brahman	1	0	0-97.5	
	Holstein	1	0	0-97.5	
District	Tshwane	138	7.2	3.5-12.9	0.213
	Sedibeng	94	8.5	0.26-5.8	
	Metsweding	4	0	0-60.2	
	West Rand	29	3.4	0.08-17.8	
	Ekurhuleni	53	3.8	0.5-13.0	
Municipality	City of Tshwane	129	6.9	3.2-12.8	0.387
	Ekurhuleni Metro	53	3.8	0.5-13.0	
	Emfuleni	37	0	0-94.8	
	Kungwini	13	7.7	0.19-36	
	Lesedi	57	1.8	0.04-9.3	
	Mogale City	29	3.4	0.08-17.8	
Abattoirs	Abattoir A	8	0	0-36.9	0.005
	Abattoir B	26	23.1	8.9-43.6	
	Abattoir C	21	4.8	0.12-23.8	
	Abattoir D	29	3.4	0.08-17.8	
	Abattoir E	28	3.6	0.09-18.3	
	Abattoir F	19	0	0-17.6	
	Abattoir G	13	7.7	0.19-36.0	
	Abattoir H	25	4	0.1-20.4	
	Abattoir I	28	3.6	0.09-18.3	
	Abattoir J	29	0	0.0-11.9	
	Abattoir K	31	0	0.0-11.2	
	Abattoir L	20	0	0.0-16.8	
	Abattoir M	24	0	0.0-14.2	
	Abattoir N	17	0	0.0-19.5	
	Abattoir O	19	10.5	1.3-33.1	
Origin of animals	Auctions	43	2.3	0.05-12.3	0.702
	Farm/Feedlot	275	4.7	2.5-7.9	
Abattoir type	HT-Multi	297	4.4	2.4-7.4	1
	LT-Multi	21	4.8	0.1-23.8	
Total	318	4.4	2.4-7.3		

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Table 6.2 Overall estimated prevalence of avian reactors in cattle in Gauteng abattoirs

Variable	Level	n	Prevalence (%)	95% CI	p-value
Species	Bovine	318	5.9	3.6-9.2	0.623
	Ovine	22	0	0-15.4	
Sex	Male	252	7.1	4.3-11.0	0.14
	Female	66	1.5	0.04-8.2	
Breed	Bonsmara	231	7.8	4.7-12.0	0.178
	Nguni	48	0	0-7.3	
	Jersey	37	2.7	0.07-14.2	
	Brahman	1	0	0-97.5	
	Holstein	1	0	0-975	
District	Tshwane	138	3,6	1.2-8.3	0.169
	Sedibeng	94	8,5	3.7-16.0	
	Metsweding	4	0	0-60.2	
	West Rand	29	13.8	3.9-31.7	
	Ekurhuleni	53	3.8	0.5-13.0	
Municipality	City of Tshwane	129	3.9	1.2-8.8	0.096
	Ekurhuleni Metro	53	3.8	0.5-13	
	Emfuleni	37	2.7	0.07-14.1	
	Kungwini	13	0	0-24.7	
	Lesedi	57	12.3	5.1-23.7	
	Mogale City	29	13.8	3.8-31.7	
Abattoirs	Abattoir A	8	12.5	0.31-52.6	0.004
	Abattoir B	26	0	0-13.2	
	Abattoir C	21	19	5.4-41.9	
	Abattoir D	29	13.8	3.9-31.6	
	Abattoir E	28	3.6	0.09-18.3	
	Abattoir F	19	0	0-17.6	
	Abattoir G	13	0	0-24.7	
	Abattoir H	25	4	0.1-20.4	
	Abattoir I	28	3.6	0.09-18.3	
	Abattoir J	29	20.7	7.9-39.7	
	Abattoir K	31	0	0.0-11.2	
	Abattoir L	20	0	0.0-16.8	
	Abattoir M	24	0	0.0-14.2	
	Abattoir N	17	5.9	0.2-28.6	
	Abattoir O	19	0	0.0-17.6	
Origin animals	Auctions	43	0	0.0-8.2	0.088
	Farm/feed lot	275	6.9	4.2-10.6	
Abattoir type	HT-Multi	297	6	3.6-9.4	1
	LT-Multi	21	4.8	0.1-23.8	
Total		318	5.9	3.6-9.1	

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Table 6.3 Overall estimated prevalence of cattle reacting to any Mycobacterium spp.

Variable	Level	n	Prevalence (%)*	95% CI	p-value
Species	Bovine	318	10	7.0-14.0	0.246
	Ovine	22	0	0-15.4	
Sex	Male	252	11.9	8.2-16.6	0.037
	Female	66	3	0.36-10.5	
Breed	Bonsmara	231	13	8.9-18	0.023
	Nguni	48	0	0-7.4	
	Jersey	37	5.4	0.7-18.2	
	Brahman	1	0	0-97.5	
	Holstein	1	0	0-975	
District	Tshwane	138	7.2	3.5-12.9	0.681
	Sedibeng	94	8.5	0.26-5.8	
	Metsweding	4	0	0-60.2	
	West Rand	29	3.4	0.08-17.8	
	Ekurhuleni	53	7.5	2.1-18.2	
Municipality	City of Tshwane	129	10.1	5.5-11.6	0.357
	Ekurhuleni Metro	53	7.5	2.1-18.2	
	Emfuleni	37	2.7	0.06-14.2	
	Kungwini	13	7.7	0.19-36.0	
	Lesedi	57	14	6.25-25.8	
	Mogale City	29	17.2	5.8-35.8	
Abattoirs	Abattoir A	8	12.5	0.31-52.6	0.063
	Abattoir B	26	23.1	8.9-43.6	
	Abattoir C	21	19	5.4-41.9	
	Abattoir D	29	17.2	5.8-35.8	
	Abattoir E	28	7.1	0.9-26.0	
	Abattoir F	19	0	0-17.6	
	Abattoir G	13	7.7	0.19-36.0	
	Abattoir H	25	8	0.1-26.0	
	Abattoir I	28	7.1	0.09-23.5	
	Abattoir J	29	20.7	7.9-39.7	
	Abattoir K	31	0	0.0-11.2	
	Abattoir L	20	0	0.0-16.8	
	Abattoir M	24	0	0.0-14.2	
	Abattoir N	17	5.8	0.1-28.7	
	Abattoir O	19	10.5	1.3-33.1	
Origin animals	Auctions	43	2.3	0.05-12.3	0.098
	Farm/feed lot	275	11.3	7.8-15.6	
Abattoir type	HT-Multi	297	10.1	6.9-14.1	1
	LT-Multi	21	9.5	1.2-30.3	
Total		318	10	7.0-13.0	

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Chapter 7: Isolation, identification and characterization of *Mycobacterium* spp. isolated from slaughtered livestock tissues and environmental samples, Gauteng province, South Africa.

7.1. Abstract

Background: *Mycobacterium tuberculosis* complex (MTBC) is a group of bacteria responsible for causing tuberculosis and infects both animals and humans. The disease results in huge economic losses estimated at billions of dollars annually. Meat inspection in slaughterhouses for the detection of lesions has been used for passive surveillance, monitoring, and diagnosis of the status of the disease. The objective of this study was to isolate, identify and characterize *Mycobacterium* spp. from slaughtered livestock tissues and environmental samples in Gauteng province of South Africa. This cross-sectional study was conducted at selected red meat abattoirs (low-throughput, high-throughput, and rural/informal) in Gauteng province, South Africa. Cattle, pigs, and sheep were sampled between the period 2017-2018. A total of 2000 tissue samples were collected at the point of slaughter from different organs (i.e. liver, lung, spleen, and different lymph nodes from each animal) from 19 red meat abattoirs, additionally, 19 environmental samples were collected from feedlots, or where animals drink water while awaiting slaughter. The samples were cultured on LJ media containing both pyruvate and glycerol and bacterial growth was monitored during weekly monitoring over 10 weeks. Bacterial growth observed during monitoring was subjected firstly to ZN staining to detect acid-fast bacteria. Acid-fast isolates were subjected to a polymerase chain reaction test (PCR) targeting *Mycobacterium tuberculosis* complex bacteria.

Results: No MTBC complex species were detected by PCR from the 32 bacterial isolates obtained from tissue cultures. The same isolates were then subjected to 16S rRNA PCR and sequence analysis to investigate and identify non-tuberculous mycobactrium species following

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Ziehl Neelsen staining of some of the isolates. Eight of the 32 isolates, from eight animals originating from four abattoirs were identified as *Mycobacterium* species by amplification of a 577 bp amplicon following gel electrophoresis. Sequence analysis of the eight isolates confirmed only two of the isolates to be each *Mycobacterium colombiense* (99.81% identity) and *Mycobacterium simiae* (99.42% identity). The remaining six isolates were identified as members of the *Actinomadura* species. From the environmental samples, isolation was made from three samples, and two could only be identified up to genus level (*Mycobacterium* species), whereas the remaining isolate was identified as *Mycobacterium senuense* (99.22% identity).

Conclusion: The study revealed the absence of bovine tuberculosis-causing pathogens in red meat abattoirs of Gauteng province, SA. For the livestock slaughtered, results suggest that there was no evident risk of transmission of bovine tuberculosis to abattoir workers and the meat was probably safe for human consumption. Although non-tuberculous *Mycobacteria* have been implicated to be potentially involved in causing tuberculosis-like diseases, their occurrence in the current study was found to be extremely low, hence insignificant.

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7.2 Introduction

Bovine tuberculosis (bTB) is a zoonotic disease with economic losses estimated at billions of dollars annually worldwide, yet the economic impact of the disease in African countries remains unquantified (Asebe, 2017). In South Africa in 2016, it was reported that wildlife species were among the tourist attraction sectors contributing 2.9% of the total Gross Domestic Product (Potgieter *et al*., 2019; Arnot & Michel, 2020) and creating 140 000 permanent jobs (Arnot & Michel, 2020). Therefore, infection of both wildlife and livestock by the disease can negatively impact the economy of the country, especially in the tourism and farming sectors (Michel *et al*., 2019). The causative agent of bTB is a bacillus that belongs to the MTBC which affects both animals and humans. Cattle are considered as the primary domestic host of the disease and African buffalo (*Syncerus caffer*)as the wildlife reservoirs of bTB. There are also a number of other species susceptible to infection with *M. bovis*, thus, there is always a high risk of spillover and spillback of *M. bovis* within livestock-wildlife-human ecosystems (Hlokwe *et al*., 2011, 2014).

As a result of the economic implications brought about by this disease, there is a growing need for the detection of tuberculous animals especially in developing countries where the surveillance and control programmes are either inadequate or not effectively implemented (Ayele *et al*., 2004, Woldemariam *et al*., 2021). Moreover, in low and middle income coutnries, the risk of zoonotic disease transmission s higher when humans and animals share resources (Nemomsa *et al*., 2014). Thus, the great concern is the zoonotic potential of the disease to public health highlighting the need for proper testing facilities for both humans and non-human animals. Meat inspection in slaughterhouses for the detection of lesions has been used for passive surveillance, monitoring, and diagnosis of the status of the disease (Woldemariam *et al*., 2021). However, it was reported that meat inspection lacks sensitivity as it can only detect lesions in only approximately 50% of infected cattle. Also, this method only detects lesions in

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advanced stages of infection when visible lesions are observed (Shitaye, 2006). Furthermore, it should be noted that lesions observed are not only due to *M. bovis*, but some members of the *Mycobacterium avium* complex (MAC) are also both pathogenic and opportunistic and may produce lesions (Dvorska *et al*., 2004) in the infected animals. Studies have suggested that meat inspection should be done together with routine culturing and followed by molecular methods for characterizing different mycobacterial species (Marfil *et al*., 2021). Moreover, molecular techniques that target the 16S rRNA for detection of non-tuberculous (NTM) are essential, especially in regions with a high TB burden and where NTMs remain undiagnosed (Gopinath & Singh, 2010). It has been reported that approximately one-third of the NTMs are responsible for disease in humans (Katoch, 2004). In the last three decades, there has been a recorded increase in NTM laboratory isolation (Johnson & Odell, 2014). In a study by Oloya *et al.* (2007) in Uganda, 19 *M. bovis* and 11 NTM isolates were isolated from 61 tissues samples from slaughter livestock, confirming widespread prevalence of the bTB in Africa. In another study, Awah-Ndukum *et al*. (2012) confirmed the presence of bTB by isolating *M. bovis* from samples collected at abattoirs. Molecular epidemiology of bTB is useful in several factors such as determination of risk factors of bTB transmissions, identification of the sources of contamination, tracking of the geographic distribution and the spread of Mycobacterial species. Molecular techniques such as PCR are considered to be more sensitive than culture. Other molecular techniques important in the differentiation and identification of strains.. Molecular deletion typing differentiates between *M. bovis* from the other strains of the MTBC. Molecular diagnostics, such as polymerase chain reaction, spoligotyping, restriction fragment length polymorphism (RFLP), variable number tandem repeats typing (VNTR), and polymorphism GC-rich repeat sequence (PGRS) are the techniques used for concurrent detection and typing of *Mycobacterium* species (Pal *et al*., 2021).

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This chapter aimed to isolate, identify and characterize *Mycobacterium* spp. from slaughtered livestock tissues and environmental samples in Gauteng province of SA. The study was conducted at functional abattoirs in the province. This study focused on determining the current infections and the risk of the disease transmission posed to abattoir workers and meat consumers.

7.3. Materials and methods

7.3.1. Description of the study area, sample type and size

This study was conducted in 19 red meat abattoirs across Gauteng province of South Africa. The sampled abattoirs consisted of 16 high-throughput (HT) and 3 low-throughput abattoirs. Gauteng is the smallest province in South Africa with an area of approximately 18 178km^2 and is considered an economic centre of the country. The province consists of 6 districts, namely, the City of Johannesburg, the City of Tshwane, Ekurhuleni, Metsweding, Sedibeng, and West Rand. The study was conducted in all 6 districts. At each selected abattoir, slaughter cattle, pigs and sheep were sampled on a single day using a systematic random sampling method and this was determined by the abattoir setting that is what we were able to sample were the animals that were slaughtered in that abattoir on that day of the visit. A total of 500 animals were sampled, i.e., cattle (n=369); pigs (n= 90) and sheep (n= 41) and 2000 samples collected. Sample size determination was done according to a formula previously described (Thrusfield *et al*., 2013). Thrusfield, M., (2007) using the formula described below:

$n_o = t^2 (p) (1-p)/ d^2$.

where t=distribution, p=prevalence (1.0%), d=precision of 1.0%) and n_o=estimated sample size. A minimum of 480 animals is required for the results to be statistically valid.

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7.3.2. Pre- and post-slaughter inspection

The professional meat inspectors assigned to each abattoir conducted pre-and post-slaughter inspections according to a standard procedure, which included the detection of clinical TB lesions. The pre-slaughter inspection was conducted by checking for abnormalities in respiration, behaviour, etc., of the animal that was being inspected. A post-slaughter inspection was performed as follows: the internal and external surfaces of the carcass were examined for the detection of any lesions. For head inspection, lymph nodes such as retropharyngeal lymph node, parotid, and submaxillary were incised and examined. In cattle, the oesophagus was separated and examined. For inspection of the viscera in sheep, the bronchi were opened, and in cattle, the larynx, trachea, and bronchi were opened and examined. The heart was incised from the base to the apex and examined. The hepatic system and the liver were also excised and examined. Other organs such as the stomach, spleen including its lymph nodes, and the gastrointestinal tract were also examined. Any part of the carcass that showed abnormalities was condemned. Biographical information about the slaughtered livestock including gender, breed, districts, and municipality origin of animals was recorded (Mareledwane *et al*., 2021).

7.3.3. Sample collection

While the inspectors were conducting physical examinations of the carcasses, the following organs were collected; *i.e*., lymph nodes (retropharyngeal, abdominal, mesenteric), liver, lung, and spleen for *Mycobacterium* species culture test. Overall, a total of 2000 tissue samples were collected from different organs (i.e. liver, lung, spleen, and different lymph nodes from each animal) from cattle (n=369), pigs (n=90), and sheep (n=41). Tissue samples were labelled and placed in sterile Ziploc bags or specimen containers. All the tissues were transported to the Tuberculosis laboratory (at ARC-Onderstepoort Veterinary Research) in ice-cooled boxes. At the laboratory, the tissue samples were cut into small pieces weighing 5 g using sterile blades. From each abattoir, water samples were collected. The samples were collected from feedlots

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or where animals drink water while waiting for slaughter. In total, 19 environmental samples were collected. All the collected environmental and tissue samples were cultured according to established standard laboratory protocols and monitored weekly for bacterial growth.

7.3.4. Isolation of *Mycobacterium* species from tissue samples

The size, smell and appearance of the tissue samples were recorded. To culture tissue samples for *Mycobacterium* spp., standard methods available in the Tuberculosis laboratory of the ARC-Onderstepoort Veterinary Research (OVR) were used. Approximately 944 samples from 236 animals were individually processed. Samples from the same animals were processed as a pool for the remaining 1056 samples (264). Briefly, the collected tissues samples were cut into approximately 5 g pieces and all the fat removed. Using a homogeniser, the samples were then homogenised. The homogenates were poured into two 50 ml tubes in preparation for the decontamination. The samples were then decontaminated in the first tube with HCL to a final concentration of 1.0% and in the second tube with NaOH to a final concentration of 2.0% for 10 minutes, followed by centrifugation (3500 rpm). The Lowenstein-Jensen (LJ) media supplemented with pyruvate (4 slopes) and glycerol (2 slopes) was inoculated with the sample pellets following neutralisation with sterile distilled water and centrifugation (3500 rpm). Pyruvate promotes the growth of MTBC species and glycerol promotes the NTMs. For each batch of samples tested, positive and negative controls were included. The positive control was obtained from a known bTB positive sample and while the negative control sample was obtained from a carcass/sample that tested negative for bTB. The inoculated media slopes were incubated at 37°C for 10 weeks and examined weekly for the presence of bacterial growth (colonies). The tissue samples were processed according to the standard operating procedures at the Tuberculosis Laboratory (Hlokwe *et al*., 2017).

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7.3.5. Isolation of *Mycobacterium* species from water samples

For isolation of non-tuberculous *Mycobacterium* species, water samples were collected from the selected abattoirs and cultured according to the standard laboratory protocol at the Tuberculosis laboratory. Positive (spiked water) and negative (unpiked sterile distilled water) controls were added for each set of samples to be tested. Briefly, 2.0% NaOH was added to the samples and centrifuged, thereafter 5.0% of oxalic acid was added. After centrifugation, Lowenstein-Jensen (LJ) slopes (3x) were inoculated with the sample and incubated at 27.0°C, and also another set was inoculated and incubated at 37.0°C, monitored for up to 10 weeks for colonies typical of *Mycobacterium species*. Thereafter, colonies were selected for Ziehl-Neelsen staining and PCR identification.

7.3.6. Ziehl-Neelsen staining and microscopy

The presence of colonies following the detection of growth on the selective media after incubation was suggestive of the presence of *Mycobacteria* and bacterial smears were prepared on microscopic slides for Ziehl-Neelsen (ZN) staining to confirm acid-fastness. The microscopic slides were placed on the rack and heat-fixed using a flame. Carbol fuchsin stain was added onto the slides and heat steamed for 5 minutes. The slides were then rinsed with water and 95% ethanol added on to them for decolourisation. After that, the slides were rinsed with water and counterstained with Malachite green. The slides were rinsed with water and left to dry. The ZN-stained smears were then observed under a microscope. Upon a positive confirmation of acid-fastness, all acid-fast colonies were subjected to a polymerase chain reaction (PCR) for further characterization.

7.3.7. DNA extraction in preparation for Polymerase Chain Reaction

DNA templates were prepared from acid-fast bacteria and individual colonies were picked up from the L-J media and mixed with 100 µl ultra-pure water. DNA was extracted from isolates

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by heat-treating the suspension at 100ºC for 25 minutes and allowed to cool down at room temperature. DNA templates were stored at -20°C until PCR analysis (Hlokwe *et al.*, 2011).

7.3.8. Identification of *Mycobacterium tuber*culosis complex species

To identify *Mycobacterium tuberculosis* complex species (MTBC), PCR assays using primers targeting the region encoding MPB 70 antigen belonging to MTBC was conducted. Briefly: a master mix consisting of ultra-pure water, buffer, 25 mM $MgCl_2$, dNTP mix (1 mM), TB 1A (20 pmol/µl), TB 1B (20 pmol/µl), 10 µl of acid-fast bacterial lysate (DNA template). The enzyme Taq polymerase (supertherm) was added and the reaction mixture was placed in a thermocycler under appropriate PCR conditions. PCR cycling conditions were as follows: Initial denaturation at 94°C for 5 minutes, denaturation at 94°C for 30 seconds, annealing at 64°C for 30 seconds, and extension at 72°C for 2 minutes for 40 cycles. The PCR products were visualized on a 1.5% agarose gel. Positive and negative controls (previously tested DNA templates) were included for quality control purposes. In addition, distilled water was included as a blank control.

Table 7.1: Components that were used for the master mix for the PCR reaction

Reagents	
Ultra-pure water	25 µl
10X reaction buffer	5 µl
25 mM $MgCl_2$	3 µl
1 mM dNTP	2.5 µl
20 pmol/µl TB 1A forward primer	2 µl
20 pmol/µl TB 1B reverse primer	2 µl
SuperTherm Taq polymerase	0.5 µl
Total volume	40 µl

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Table 7.2 Primers that were used for the PCR procedure

Forward primer	(5′ GAACAATCCGGAGTTGACAA 3′)
Reverse primer	(5′ AGCACGCTGTCAATCATGTA 3′)

7.3.9. Identification of NTMs by PCR and sequence analysis

Non-tuberculous mycobacteria (NTM) were identified by PCR and sequence analysis of the 577 bp of the *Mycobacterium* 16S rRNA gene (Gcebe *et al*., 2013). The mycobacterial cell lysate was used as a DNA template and the PCR cycling parameters were as follows: initial denaturation at 95 °C for 15 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and elongation at 72 °C for 30 s and a final extension at 72 °C for 10 min. The amplicons were sent to Inqaba Biotechnical Industries, Ltd, South Africa for sequencing of the forward 16S rRNA gene using an ABI sequencer. Sequences were edited manually and pairwise alignments were undertaken using the BioEdit Sequence alignment editor (version 7.1.9). The sequences were analyzed on the NCBI BLAST platform for species identification by the mega blast.

Table 7.3 Primers that were used for the 16S rRNA PCR procedure

16S rRNA forward	5′ AGA GTT TGA TCC TGG CTC AG 3′
16S rRNA – Reverse	5′ GCG ACA AAC CAC CTA CGA G 3′

7.3.10. Ethical considerations

The study was approved by the Animal Ethics Committees for the Agricultural Research Council-Onderstepoort Veterinary Research (AEC 12.16) (Appendix 5.2) and the University of Pretoria (V104-17) (Appendix 5.3). Authorization to carry out the study at the abattoir was

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granted by the Department of Agriculture, Land Reforms and Rural Development (DALRRD) through section 20 approval (Appendix 5.4).

7.4. Results

7.4.1. Bacterial isolation (culture) and Ziehl Neelsen staining from tissue samples

Tissue samples originating from 19 different red meat abattoirs located in the Gauteng province were collected and tested at the Tuberculosis Laboratory at the Agricultural Research Council (ARC-OVR) for *Mycobacterium* spp isolation. Colony-shaped bacterial growth typical of mycobacteria was observed. Culture results from colonies resembling mycobacterium species which were isolated are summarised in Table 7.1. Isolation (single colonies per sample represented by 6 slopes) was made from 32 samples representing animals from 12 different red meat abattoirs.

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Table 7.4: Bacterial isolation from samples collected from animals originating from different abattoirs.

Abattoir	Animal number (from which isolation was successsful)
1. Klipeiland	Isolations made from animals: 2,3,4,5,11,12 and 14
2. Farmers' Meat	Isolations were made from animals: 4, 13 and 18
3. Comet	Isolations were made from animals 1, 2, 3, 6, 8 and 14
4. PK	Isolations were made from animal 1 and 21
5. Pork Parkers	Isolation was made from animal 21
6. Boschkop	Isolation was made from animal 14
7. Kameeldrift	Isolation was made from animal 8
8. Vereeniging	Isolation was made from animal 8
9. Morgan Beef	Isolations were made from animals 13 and 17
10. Strydfontein	Isolations were made from animals 7,9,19 and 27
11. Chalmar Lamb	Isolations were made from animal 18 and 20
12. Chalmar Beef	Isolations were made from animals 25 and 26

Acid-fast bacilli were only identified following Ziehl-Neelsen and microscopic examinations of the bacterial smears prepared from colonies that had morphology resembling that of mycobacteria. From the ZN staining and microscopic examination, acid-fast rods were observed from Animal 3 originating from Klipeiland abattoir, Animals 4 and 18 from Farmers Meat abattoir (Figure 7.1). The majority of the colonies were too small to conduct ZN smears.

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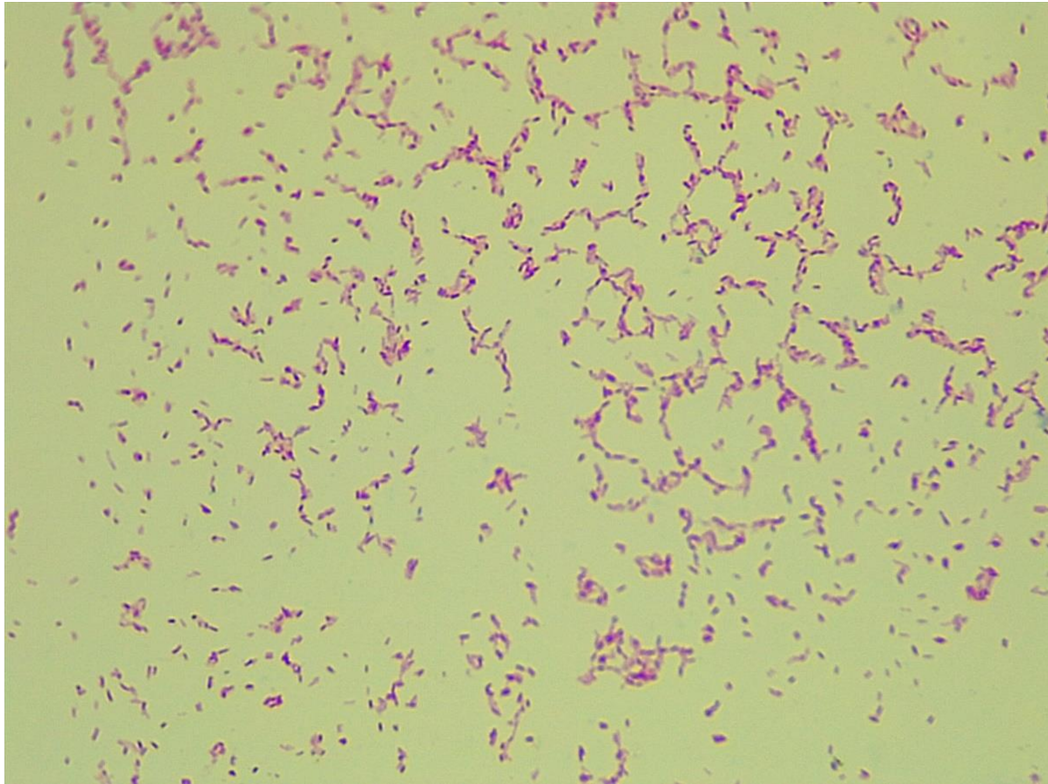

Figure 7.1: Example of a Ziehl-Neelsen staining results of acid-fast rods isolated from one of the abattoirs.

7.4.2.1. Identification of *Mycobacterium tuberculosis* complex species

Thirty-two isolates that had colonies that displayed morphology resembling that of *Mycobacteria* were subjected to TB 1 AB PCR (targeting *Mycobacterium tuberculosis* complex bacteria). Out of the 32 isolates (Figure 7.2), there was no MTBC complex isolated.

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Figure 7.2: Example of a gel electrophoresis results obtained from TB 1AB PCR for identification of *Mycobacterium tuberculosis* complex bacteria. Lane M is a 100 bp DNA marker; Lanes 1-7 represents isolates from Klipeiland and lane 8-10 represents isolates from Farmers Meat market. Lane + represent positive control (TB 9845A), Lane MOTT (TB 9801B) represents a negative non-target control and Lane - represents the negative blank control (distilled water).

7.4.2.2. Characterization of NTM isolates from tissue samples

An amplicon with size approximately of 577 bp was obtained and indicated the presence of *Mycobacteria*. Out of the 32 isolates, eight isolates from eight animals originating from four abattoirs were identified as *Mycobacterium* species by 16S rRNA (Figure 7. 3). Sequence data of the eight isolates subjected to 16S rRNA gene analysis identified only two of the isolates as mycobacteria, i.e.; *Mycobacterium colombiense* (99.81% identity) isolated from animal # 18, which originated from Farmers' Meat abattoir; and *Mycobacterium simiae* (99.42%) isolated

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from animal # 27 sampled at Strydfontein abattoir. The remaining six isolates were identified as members of the *Actinomadura* species.

Sequence shows 99.81% similarity to *Mycobacterium colombiense*

GGGGGGGGCCTTTAAcCATGCAGTCGAACGGAAAGGCCTCTTCGGAGGTA
CTCGAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTTC
GGGATAAGCCTGGGAAACTGGGTCTAATACCGGATAGGACCTTTAGACGC
ATGTCTTTTGGTGGAAAGCTTTTGCGGTGTGGGATGGGCCCGCGGCCTAT
CAGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGC
CTGAGAGGGTGTCCGGCCACACTGGGACTGAGATACGGCCCAGACTCCTA
CGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAG
CGACGCCGCGTGGGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCACCA
TCGACGAAGGTCCGGGTTTTCTCGGATTGACGGTAGGTGGAGAAGAAGCA
CCGGCCAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTT
GTCCGGAATTACTGGGCGTAAAGAGCTCGTAGGTGGTTTGTCGC

Sequence shows 99.42% similarity to *Mycobacterium simiae*

CGTGCGGGGGCCTTAMCATGCAAGTCGAGCGGAAAGGCCCCTTCGGGGGT
ACTCGAGCGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCCTGACTC
TGGGATAAGCCCGGGAAACTGGGTCTAATACCGGATATGACGCACTCTCG
CATGGGATGTGTGTGGAAAGTTTTTCGGTTGGGGATGGGCTCGCGGCCTA
TCAGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAACCGG
CCTGAGAGGGCGACCGGTCACACTGGGACTGAGACACGGCCCAGACTCCT
ACGGGAGGCAGCAGTGGGGAATATTGCGCAATGGGCGGAAGCCTGACGCA
GCGACGCCGCGTGGGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGC
AGGGACGAAGCTAACGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTA
CGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTA
TT

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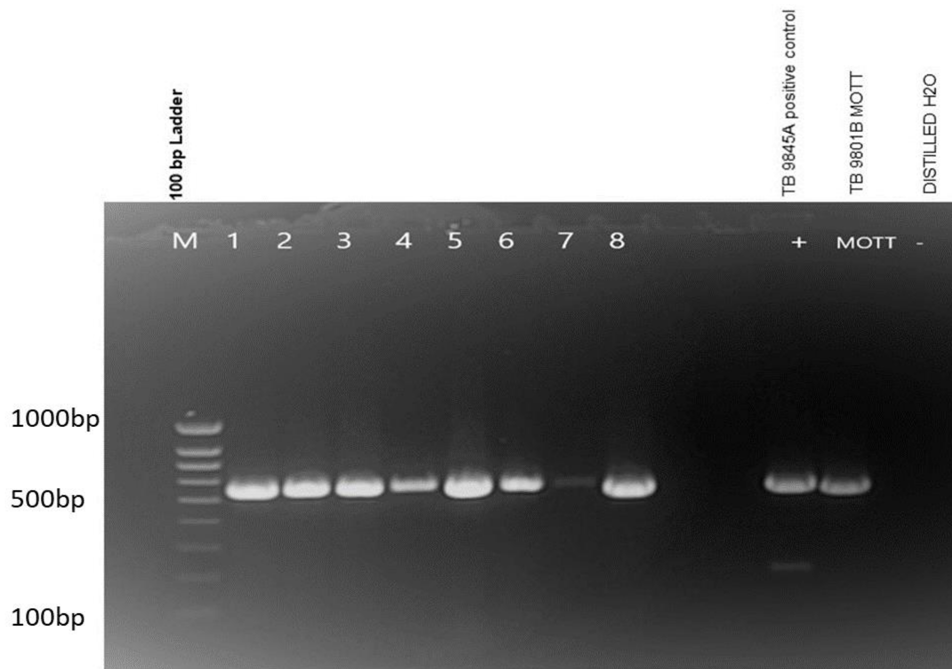

Figure 7.3: Gel electrophoresis results obtained from 16S rRNA gene analysis. Lane M is 100 bp DNA marker; Lane 1 represents an isolate from abattoir N (Klipeiland), Lanes 2-4 represent isolates from abattoir P (Farmers Meat), Lanes 5-7 represent an isolate from abattoir B (Comet), Lane 4 represent an isolate from abattoir P, Lane 5 represent an isolate from abattoir B while Lane 8 represents an isolate from abattoir K (Strydfontein), Lane - represent positive control (TB 9845A), Lane MOTT represents negative non-target control (TB9801B) and Lane – represents the negative control which was distilled water.

7.4.3. Isolation of *Mycobacteria* from environmental samples

Isolates from five abattoirs: Diamond L, Farmers Meat, Irene, PK, and Vereeniging were confirmed to be acid-fast. Figure 7.4 shows the acid-fast isolates that were confirmed by Ziehl-Neelsen staining.

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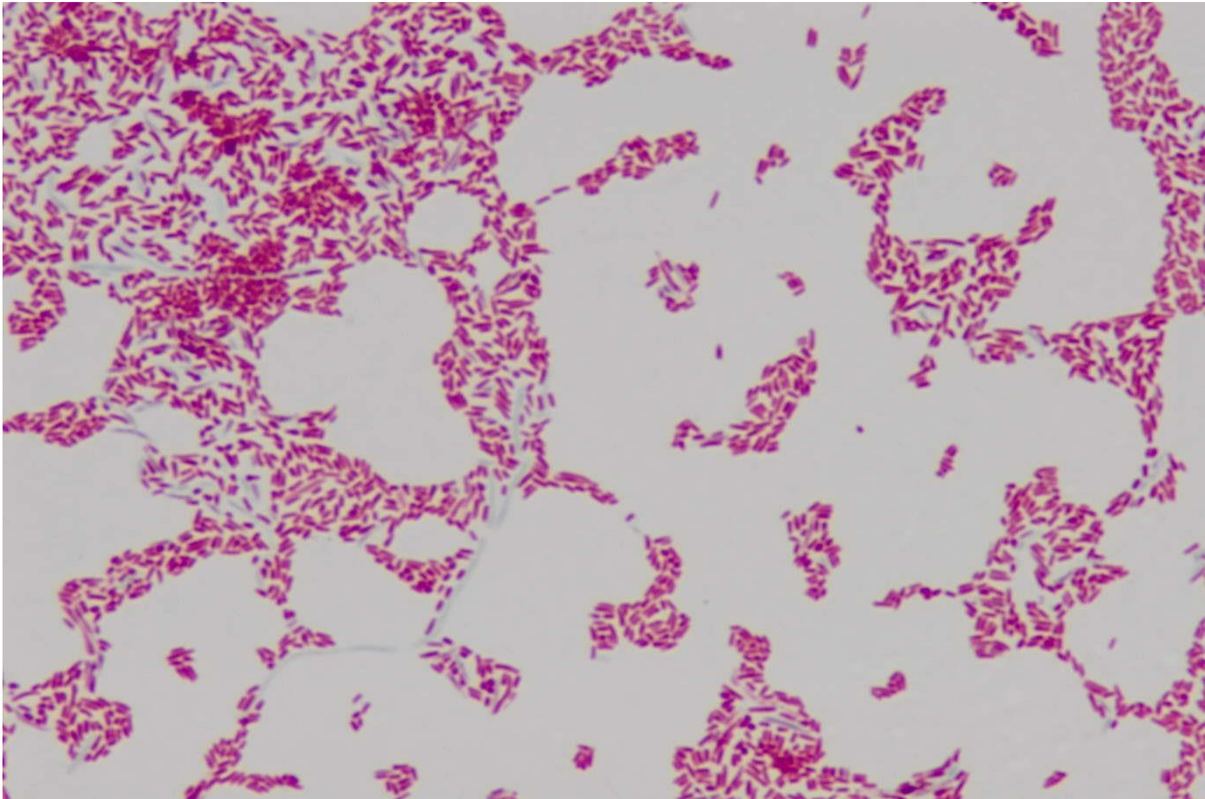

Figure 7.4: Example of Ziehl-Neelsen staining of acid-fast rods isolated from one of the abattoirs.

7.4.3.1. Characterization of NTM isolates from environmental isolates

Out of the five isolates prepared (Diamond L, Irene, PK, and Vereeniging), we obtained specific amplification from four of the isolates (15.8%). The expected size of the PCR product was approximately 577 bp (Figure 7.5). The fourth isolate from the Farmers' Meat abattoir, the concentration for this isolate was too low and therefore it could not be submitted for sequencing at Inqaba Biotechnical Industries, Ltd, South Africa. The three PCR products from PK, Diamond L, and Vereeniging were sent for sequencing. Sequence data analysis identified one of the isolates up to genus level only the isolates (*Mycobacterium* species at 98.89% identity). This isolate was from a sample collected at the PK abattoir. *Mycobacterium avium* (99.61% identity) was isolated from a sample collected at the Diamond L abattoir, and *Mycobacterium senuense* (99.02% identity) from Vereeniging abattoir.

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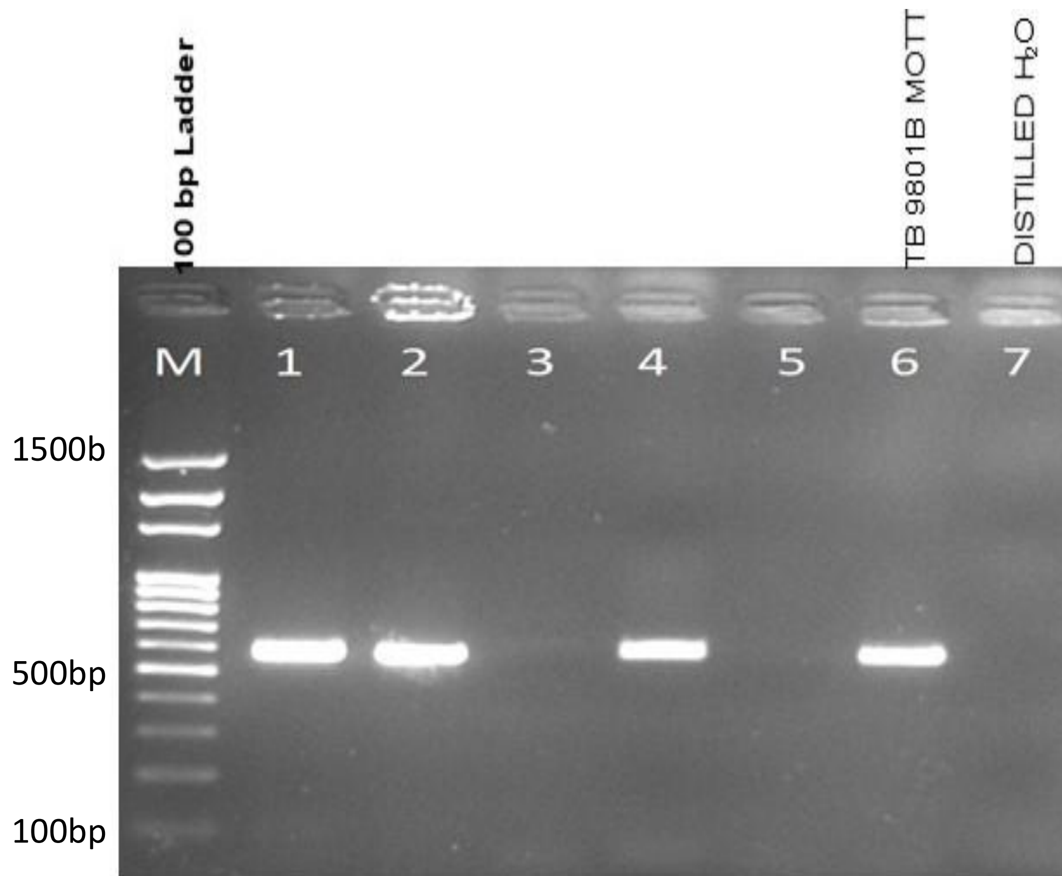

Figure 7.5: Gel electrophoresis results obtained from 16S gene analysis. Lane M is a 100 bp DNA marker; Lane 1, 2, 3, 4, 5 represent isolates from abattoirs PK, Diamond L, Farmers' Meat, Vereeniging abattoir, and Irene, Lane 6 represents positive control (MOTT), Lane 7 represents the negative control (sterile distilled water).

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Sequence shows similarity 98.89% to *Myobacterium* spp.

TGggaTTTACACATGCAAGTCgAACGGAAAGGCCCCTTCgGGGTGCTCG

AGTGGCGAACGgGTGAGTAACACGTGGGTGATCTGCCCTGCACTCTGGGA

TAAGCTTGGGAAACTGGGTCTAATACCGGATAGGACCATGGGATGCATGT

TCTGTGGTGGAAAGCTTTTGCGGTGTGGGATGGCCCGCGGCCTATCAGC

TTGTTGGtGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGA

GAGGGTGTCCGGCCACACTGGGACTGAGATACGGCCCAGACTCCTACGGG

AGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCGAC

GCCGCGTGGGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGTATCGG

CGAAGCTCCGTGGTTTTCTGCGGGGTGACGGTAGGTACAgAAgAAGCACC

GGCCAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTGT

CCGGAATTACTGGGCGTAAAGAGCTCGTAGGTGGTTTGTCgCA

Sequence shows 99.61% similarity to *Mycobacterium avium*

CGgGGgGCTTACacATGCAGTCGAACGGAAAGGCCTCTTCGGAGGTACTC

GAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTTCGGG

ATAAGCCTGGGAAACTGGGTCTAATACCGGATACGACCTTTAGACGCATG

TCTTTTGGTGGAAAGCTTTTGCGGTGTGGGATGGGCCCGCGGCCTATCAG

CTTGTTGGtGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTG

AGAGGGTGTCCGGCCACACTGGGACTGAGATACGGCCCAGACTCCTACGG

GAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCGA

CGCCGCGTGGGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCACCATCG

ACGAAGGTTCGGGTTTTCTCGGATTGACGGTAGGTGGAGAAGAAGCACCG

GCCAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTGTC

CGGAATTACTGGGCGTAAAGAGCTCGTAGGTGGTTTGTCgCA

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Sequence shows 99.02% similarity to *Mycobacterium senuense*

AgGTgaCTTAcACATGCAAGTCGAACGGAAAGGCCCTTTCGGGGGTGCTC

GAGTGGCGAACGGGTGAGTAACACGTGGGTGATCTGCCCTGCACTCTGGG

ATAAGCCTGGGAAACTGGGTCTAATACCGGATAGGACCATGGGATGCATG

TTCTGTGGTGGAAAGCTTTTGCGGTGTGGGATGGGCCCGCGGCCTATCAG

CTTGTTGGtGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTG

AGAGGGTGTCCGGCCACACTGGGACTGAGATACGGCCCAGACTCCTACGG

GAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCGA

CgCCGCGTGGGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGTATCG

GCGAAGCTCCCGGATTTTTCTGGGGGTGACGGTAGGTACAgAAgAAGCAC

CGGCCAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTG

TCCGGAATTACTGGGCGTAAAGAGCTCGTAGGTGGTTTGTCGCA

GGGCGTAAAGAGCTCGTAGGTGGTTTGTCGCA

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7.5. Discussion

In this study, 2000 tissues samples were collected from slaughtered livestock. Approximately 944 samples from 236 animals were individually processed. For the remaining 1056 samples samples were processed as a pool (pooling was done from organs of the same animals). Hence, the study decided to pool the remaining samples from 264 animals (1056 samples) as it is proven that isolations could be made from both pooled and individually processed samples. Isolation from pooled samples is possible as previously observed in the TB lab (Hlokwe *et al.*, 2011, Hlokwe *et al.*, 2014).

In this study, no MTBC species were isolated, and it should be noted that there were no visible lesions resembling *M. bovis* infection on any of the samples processed. Tuberculosis-like lesions are an indicator of the presence of tuberculosis (Biet *et al.*, 2005; Kriek *et al.*, 2019). According to Botha *et al.* (2013), tuberculosis is a slowly and progressive disease and remains asymptomatic for a long period until during the advanced stages of infection when lesions appear. This may have been the case in this study that when livestock are taken to the abattoirs for slaughter the animals could still be in the early stages of infection and bacterial concentrations are too low to be detected by culture. In South Africa the implementation of the national bovine tuberculosis programme especially in commercial cattle resulted in decreased prevalence of bovine tuberculosis to 0.4% (Michel *et al.*, 2008). However, in countries such as Zambia, Uganda and Ethiopia herd prevalence of up to 50% have been reported in cattle (Oloya *et al.*, 2007; Munyeme *et al.*, 2009; Dejene *et al.*, 2016).

Other abattoir-based studies in slaughtered livestock in Gauteng red meat abattoirs have used a similar approach to successfully isolate zoonotic pathogens in red meat abattoirs of Gauteng province, SA. These include studies by Kolo *et al.* (2019), who successfully isolated *Brucella* spp., Dongonyaro *et al.* (2020) isolated *Leptospira* and Mangena *et al.* (2021) isolated *Coxiella burnetii*. In our previous study conducted in parallel with the current study, Mareledwane *et*

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al. (2021) reported an estimated bTB prevalence of 4.4% in cattle indicating past exposure or detection of early infection, an advantage of the cell-mediated immune response based tests. Although a 0.0% prevalence for MTBC species was recorded in the current study, *Mycobacteria* spp were isolated. Non-tuberculous mycobacteria isolated include *Mycobacterium colombiense*, *Mycobacterium simiae*, *Mycobacterium avium* and *Mycobacterium senuense*. One of the NTM isolated in this study, *M. simiae,* has been shown to have pathological conditions, especially in humans, and has been isolated more especially in patients who are immunocompromised. Infection by this NTM leads to extrapulmonary infections (Hamieh *et al*., 2018). This is one of the few studies to demonstrate the isolation of NTMs from slaughtered cattle in red meat abattoirs in Gauteng province of SA. In a previous study, Hlokwe and co-workers isolated *Mycobacterium nonchromogenicum* and an NTM species closely related to *Mycobacterium moriokaense* from cattle on two different farms in the Eastern Cape province of South Africa. The same as the outcome of the current study, these NTM species were detected from samples without visible lesions, hence their clinical significance could not be determined (Hlokwe *et al*., 2017). Nuru and co workers (2017), conducted a study in Bahir Dar abattoir in Ethiopia (Nuru *et al*., 2017). From a total of 2846 organ samples collected in abattoirs, *Mycobacterium fortuitum-peregrinum* was isolated from 6 isolates collected from cattle, while Oloya *et al*. (2007) successfully isolated *M. avium* species from slaughter cattle in Uganda.

In Africa, the diagnosis of the NTM is overshadowed by the high incidence of *M. bovis* and this results in less focus being put on the detection and isolation of NTM (Botha *et al*., 2013). As was the case in this study, where we isolated NTM both from both tissue and water samples. The fact that NTM can be found in between the environment and animals suggests that NTMs are interchangeable between the two (Gcebe *et al*., 2013). The use of molecular techniques that target the 16S rRNA gene has brought insight into the diagnosis of NTM.

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7.6. Conclusion

There is a very low prevalence of *Mycobacterium* species in Gauteng red meat abattoirs as confirmed by culture test, hence low risk posed to meat consumers. However, more attention needs to be paid to NTM isolated from abattoirs, as their role in disease is not yet well understood.

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7.7. References

Arnot, L.F. and Michel, A., 2020. Challenges for controlling bovine tuberculosis in South Africa. *Onderstepoort Journal of Veterinary Research*, *87*(1), pp.1-8.

Asebe, G., 2017. Characterization of *Mycobacterium bovis* and its significance: role for control of zoonotic tuberculosis in Africa. *Journal of Medical Diagnostic Methods*, 6(3), pp. 1-10.

Awah-Ndukum, J., Kudi, A.C., Bradley, G., Ane-Anyangwe, I., Titanji, V.P.K., Fon-Tebug, S. and Tchoumboue, J., 2012. Prevalence of bovine tuberculosis in cattle in the highlands of Cameroon based on the detection of lesions in slaughtered cattle and tuberculin skin tests of live cattle. *Veterinarni Medicina*, *57*(2), p.59.

Ayele, W.Y., Neill, S.D., Zinsstag, J., Weiss, M.G. and Pavlik, I., 2004. Bovine tuberculosis: an old disease but a new threat to Africa. *The International Journal of Tuberculosis and Lung Disease*, *8*(8), pp.924-937.

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

Botha, L., Gey van Pittius, N.C. and Van Helden, P.D., 2013. Mycobacteria and disease in southern Africa. *Transboundary and Emerging Diseases*, *60*, pp.147-156.

Cleaveland, S., Mlengeya, T., Kazwala, R.R., Michel, A., Kaare, M.T., Jones, S.L., Eblate, E., Shirima, G.M. and Packer, C., 2005. Tuberculosis in Tanzanian wildlife. *Journal of Wildlife Diseases*, *41*(2), pp.446-453.

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Dejene, S.W., Heitkönig, I.M., Prins, H.H., Lemma, F.A., Mekonnen, D.A., Alemu, Z.E., Kelkay, T.Z. and de Boer, W.F., 2016. Risk factors for bovine tuberculosis (bTB) in cattle in Ethiopia. *PLoS One*, *11*(7), p.e0159083.

Dogonyaro, B.B., van Heerden, H., Potts, A.D., Kolo, B.F., Lotter, C., Katsande, C., Fasina, F.O., Ko, A.I., Wunder, E.A. and Adesiyun, A.A., 2020. Seroepidemiology of Leptospira infection in slaughtered cattle in Gauteng province, South Africa. *Tropical Animal Health and Production*, *52*(6), pp.3789-3798.

Dvorska, L., Matlova, L., Bartos, M., Parmova, I., Bartl, J., Svastova, P., Bull, T.J. and Pavlik, I., 2004. Study of *Mycobacterium avium* complex strains isolated from cattle in the Czech Republic between 1996 and 2000. *Veterinary microbiology*, *99*(3-4), pp.239-250.

Gcebe, N., Michel, A.L. and Hlokwe, T.M., 2018. Non-tuberculous Mycobacterium species causing mycobacteriosis in farmed aquatic animals of South Africa. *BMC Microbiology*, *18*(1), pp.1-11.

Gcebe, N., Rutten, V., Gey van Pittius, N.C. and Michel, A., 2013. Prevalence and Distribution of Non-Tuberculous Mycobacteria (NTM) in Cattle, African Buffaloes (S yncerus caffer) and their Environments in South Africa. *Transboundary and Emerging Diseases*, *60*, pp.74-84.

Gopinath, K. and Singh, S., 2010. Non-tuberculous mycobacteria in TB-endemic countries: are we neglecting the danger? *PLoS Neglected Tropical Diseases*, *4*(4), p.e615.

Hamieh, A., Tayyar, R., Tabaja, H., EL Zein, S., Bou Khalil, P., Kara, N., Kanafani, Z.A., Kanj, N., Bou Akl, I., Araj, G. and Berjaoui, G., 2018. Emergence of Mycobacterium simiae: A retrospective study from a tertiary care center in Lebanon. *PloS one*, *13*(4), p.e0195390.

© University of Pretoria

Hlokwe, T. M., Jenkins, A. O., Streicher, E. M., Venter, E. H., Cooper, D., Godfroid, J., & Michel, A. L. 2011. Molecular characterisation of *Mycobacterium bovis* isolated from African buffaloes (*Syncerus caffer*) in Hluhluwe-iMfolozi Park in KwaZulu-Natal, South Africa. *Onderstepoort Journal of Veterinary Research 78: 1-6.*

Hlokwe, T.M., Van Helden, P. and Michel, A.L., 2014. Evidence of increasing intra and inter-species transmission of Mycobacterium bovis in South Africa: are we losing the battle? *Preventive Veterinary Medicine*, *115*(1-2), pp.10-17.

Hlokwe, T.M., Said, H. and Gcebe, N., 2017. *Mycobacterium tuberculosis* infection in cattle from the Eastern Cape Province of South Africa. *BMC veterinary research*, *13*(1), pp.1-9.

Johnson, M.M. and Odell, J.A., 2014. Nontuberculous mycobacterial pulmonary infections. *Journal of Thoracic Disease*, *6*(3), p.210.

Kriek, N.P., Areda, D.B. and Dibaba, A.B., 2019. The diagnosis of bovine tuberculosis. In *Tuberculosis in animals: An African perspective* (pp. 171-235). Springer, Cham.

Kolo, F.B., Adesiyun, A.A., Fasina, F.O., Katsande, C.T., Dogonyaro, B.B., Potts, A., Matle, I., Gelaw, A.K. and Van Heerden, H., 2019. Seroprevalence and characterization of Brucella species in cattle slaughtered at Gauteng abattoirs, South Africa. *Veterinary Medicine and Science*, *5*(4), pp.545-555.

Mangena, M., Gcebe, N., Pierneef, R., Thompson, P.N. and Adesiyun, A.A., 2021. Q Fever: Seroprevalence, risk factors in slaughter livestock and genotypes of *Coxiella burnetii* in South Africa. *Pathogens*, *10*(3), p.258.

Mareledwane, V.E., Adesiyun, A.A., Thompson, P.N. and Hlokwe, T.M., 2021. Application of the gamma-interferon assay to determine the prevalence of bovine tuberculosis in slaughter livestock at abattoirs in Gauteng, South Africa. *Veterinary Medicine and Science*, Jun 15.

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doi: 10.1002/vms3.492.

Marfil, M.J., Garbaccio, S.G., Barandiaran, S., Huertas, P.S., Vivot, M.M., Eirin, M.E. and Zumárraga, M.J., 2021. Isolation of Nontuberculous Mycobacteria from Bovine Raw Lungs Bought in Butchers' Shops. *Foodborne Pathogens and Disease*, *18*(11), pp.805-811.

Michel, A.L., 2008. Mycobacterium fortuitum infection interference with Mycobacterium bovis diagnostics: natural infection cases and a pilot experimental infection. *Journal of Veterinary Diagnostic Investigation*, *20*(4), pp.501-503.

Michel, A.L., Sibanda, D.R. and de Klerk-Lorist, L.M., 2019. BTB Control Strategies in Livestock and Wildlife in South Africa. In *Tuberculosis in animals: An African perspective* (pp. 387-401). Springer, Cham.

Munyeme, M., Muma, J.B., Samui, K.L., Skjerve, E., Nambota, A.M., Phiri, I.G.K., Rigouts, L. and Tryland, M., 2009. Prevalence of bovine tuberculosis and animal level risk factors for indigenous cattle under different grazing strategies in the livestock/wildlife interface areas of Zambia. *Tropical Animal Health and Production*, *41*(3), pp.345-352.

Muyoyeta, M., de Haas, P.E., Mueller, D.H., van Helden, P.D., Mwenge, L., Schaap, A., Kruger, C., Gey van Pittius, N.C., Lawrence, K., Beyers, N. and Godfrey-Faussett, P., 2010. Evaluation of the Capilia TB assay for culture confirmation of Mycobacterium tuberculosis infections in Zambia and South Africa. *Journal of Clinical Microbiology*, *48*(10), pp.3773-3775.

Meiring, C., Van Helden, P.D. and Goosen, W.J., 2018. TB control in humans and animals in South Africa: a perspective on problems and successes. *Frontiers in Veterinary Science*, p.298.

© University of Pretoria

Nemomsa, B., Gebrezgabiher, G., Birhanu, T., Tadelle, H., Tadesse, G. and Getachew, B., 2014. Epidemiology of bovine tuberculosis in Butajira, Southern Ethiopia: A cross-sectional abattoir-based study. *African Journal of Microbiology Research*, *8*(33), pp.3112-3117.

Nuru, A., Zewude, A., Mohammed, T., Wondale, B., Teshome, L., Getahun, M., Mamo, G., Medhin, G., Pieper, R. and Ameni, G., 2017. Nontuberculosis mycobacteria are the major causes of tuberculosis like lesions in cattle slaughtered at Bahir Dar Abattoir, northwestern Ethiopia. *BMC Veterinary Research*, *13*(1), pp.1-6.

Oloya, J., Muma, J.B., Opuda-Asibo, J., Djønne, B., Kazwala, R. and Skjerve, E., 2007. Risk factors for herd-level bovine-tuberculosis seropositivity in transhumant cattle in Uganda. *Preventive Veterinary Medicine*, *80*(4), pp.318-329.

Shitaye, J. E., Getahum, B., Alemayehu, T., Skoric, M., Treml, F., Fictum, P., Vrbas, V. & Pavlik, I. 2006. A prevalence study of bovine tuberculosis in cattle in different farming sysytems in the Eastern zone of Tanzania. *Preventative Veterinary Medicine,* 57(), pp.162-172

Pal, M., Berhanu, G., Diba Feyisa, B.M. and Kandi, V., 2021. Bovine Tuberculosis: A Review of Molecular Diagnostic Methods and Impact on Public Health. *American Journal of Microbiological Research*, *9*(1), pp.1-8.

Potgieter, A., Campus, G., Berman, G.K. and Verity, J.M., 2019. The relationship between socio-cultural impacts of a township tour and the overall life satisfaction of residents in townships in the Western Cape, South Africa.

Thrusfield, M., 2013. Presenting numerical data. *Veterinary epidemiology, 4rd ed. Blackwell Science Ltd., Oxford, UK*, pp.214-227.

© University of Pretoria

van Helden, P.D., van Pittius, N.C.G., Warren, R.M., Michel, A., Hlokwe, T., Morar, D., Godfroid, J., Du Plessis, E.C. and Bengis, R., 2008. Pulmonary infection due to Mycobacterium goodii in a spotted hyena (Crocuta crocuta) from South Africa. *Journal of Wildlife Diseases*, *44*(1), pp.151-154.

Woldemariam, T., Pal, M., Zewude, A., Mamo, G. and Gutama, G.A.K.P., 2021. A study on the prevalence of tuberculosis in cattle at selected abattoirs in Ethiopia. *Journal in Research Microbiology*, 2(2), pp. 9-13

Zakham, F., Bazoui, H., Akrim, M., Lemrabet, S., Lahlou, O., Elmzibri, M., Benjouad, A., Ennaji, M.M. and Elaouad, R., 2012. Evaluation of conventional molecular diagnosis of Mycobacterium tuberculosis in clinical specimens from Morocco. *The Journal of Infection in Developing Countries*, *6*(01), pp.40-45.

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Chapter 8. General discussion and concluding remarks

The study determined the prevalence and characteristics of *Mycobacterium* species in slaughter animals at Gauteng province abattoirs and to assess the risk of zoonotic tuberculosis posed to abattoir workers. Red meat abattoirs were targeted for this study because they play a very important role in disease surveillance and monitoring and have previously provided means to assess the prevalence of bTB in slaughter animals (Renwick *et al*., 2007). Generally, reports on bTB in Gauteng abattoirs in SA are limited and the study aimed at addressing this situation.

The study was conducted in four parts. Firstly, there is no recent review of diagnostic laboratory data on culturing of *Mycobacteria* spp. in SA. This prompted us to conduct a retrospective study on the samples submitted at the ARC-OVR-Tuberculosis laboratory (2007-2016) to ascertain the history and transmission of the disease in SA. The diagnostic laboratory records were analysed to identify factors associated with the transmission of this zoonosis. The analysis demonstrated an overall prevalence of 18.47% [834/4516; 95% CI: 17.37-19.63] for *Mycobacterium* spp. Risk analysis showed the presence of wildlife-livestock and human interface is a major risk factor for transmission and spread of bTB in the country. These findings are consistent with published reports (Hlokwe *et al.,* 2011; Sichewo *et al*., 2020). In addition, animals sharing resources such as water points can become contaminated with the pathogen and thus serve as a source of infection to other susceptible animals that share that water point (Humblet *et al*., 2009).

We further reviewed serological laboratory data from 2011-2016. After the laboratory data were collected from the records and analysed, the findings showed that 1.54% (95% CI: 1.30 – 1.78) of the samples tested were positive for bTB by serology using the Bovigam® kit. Avian reactors accounted for 3.75% (95% CI: 3.38 - 4.11) of the samples whereas a combination of multiple and equal reactors, as well as animals that had a positive screening test result,

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constituted 2.29%. The findings demonstrated a low prevalence of bTB and this agrees with the findings of the study conducted by Michel (2008). It was thus concluded to obtain a better picture of the status of bTB, serological data should be combined with bacteriological data.

Secondly, we conducted interviews at selected red meat abattoirs in Gauteng province, to assess the knowledge, attitude, and practices (KAP) of abattoir workers regarding tuberculosis. The study was conducted because KAP studies on TB in South Africa are few, particularly in red meat abattoirs in Gauteng province. The most recent KAP study on bTB in SA was reported by Marange *et al*. (2020), however, this was done in Mpumalanga province, South Africa, and it was not abattoir-based. In the current study, we found that 88.35% of the participants had knowledge of bTB with an overall knowledge score of 42% (Cl: 37.48 - 46.42; P<0.001). However, this is a higher frequency of knowledge of bTB compared to findings by Marange *et al*. (2020) who reported a bTB awareness of 61.0% in Mpumalanga province. The current study was able to identify risk factors such as knowledge gaps and practices that red meat abattoir workers engaged in that put them at risk of infection with *M. bovis*.

Thirdly, we conducted serological tests as to detect bTB using gamma interferon (IFN-γ) assay in red meat abattoirs of Gauteng province of South Africa. A bTB prevalence of 4.4% (95% Cl: 2.4-7.3%) was detected in cattle, while that of avian reactors was 6.0% (95% Cl: 3.6-9.2%). Sichewo *et al.,* in 2019 reported a bTB prevalence in cattle in communal herds to be 12%, a much higher prevalence than the one determined in this study where the focus was on abattoir animals. In the current study, although the prevalence of bTB was low, the outcome of the study did show that bTB was present in red meat abattoirs. The study demonstrated that additional methods such as using the IFN-γ assay would be useful in determining the infection status of animals in red meat abattoirs.

Fourthly, we also collected corresponding tissues from the sample slaughtered animals for culturing to isolate and characterize various *Mycobacterium* species. No MTBC species were

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isolated, however, two NTMs; *Mycobacterium colombiense* (identity 99.81%) and *Mycobacterium simiae* (identity 99.42%) were successfully isolated and the 16S rRNA gene sequences analyzed. Although non-tuberculous mycobacteria have been implicated to be potentially involved in causing tuberculosis-like diseases, their rate of occurrence in the current study was extremely low, hence insignificant.

In conclusion, this was the first abattoir-based study on red meat abattoirs in Gauteng province of South Africa. The entire work consisted of two retrospective studies, one questionnaire and two prevalence studies (serological and culture). The work has provided baseline data in terms of the current prevalence of mycobacterial species at the animal-wildlife-human interphase in South Africa. Although minimal, we managed to establish the potential risk posed by bTB to both livestock, and abattoir workers. In this study the prevalence of *Mycobacterium* in red meat abattoirs using the interferon-gamma assay was determined. The study highlighted the importance of continuous disease surveillance with respect to bovine tuberculosis and public health. The work demonstrated that performing surveillance at abattoirs is a good tool for monitoring prevalence of bovine tuberculosis in cattle and this lays a foundation for future epidemiological abattoir studies, hence more studies are required in red meat abattoirs in South Africa as a whole.

Recommendations

- Use of serological assays such as the interferon-gamma (IFN-γ) assay can be used as an ancillary test for detection of early signs of tuberculosis infections in abattoir settings.
- For veterinary laboratories and veterinary authorities, improved data collection and record-keeping is necessary in disease management and identification, particularly bTB and other zoonoses.

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- The South African Veterinary Council, abattoir management should ensure that public awareness campaigns are conducted on diseases of public health concern and their role in the prevention and control of such diseases should be conducted frequently, especially for high disease occupations like abattoir workers and veterinarians.

Limitations

- The laboratory data may be biased since samples that were submitted for testing focused on animals that showed clinical signs (although not always the case with bTB) or animals tested before transportation to facilities within and outside the country. The implication is that the actual prevalence of the disease may be under-reported.
- The questionnaire did not contain questions that focused on perceptions of the abattoir workers on tuberculosis, therefore information on this aspect could not be obtained.
- For the interferon-gamma assay blood samples collected must be processed within hours

9. References

Bhembe, N.L., Jaja, I.F., Nwodo, U.U., Okoh, A.I. and Green, E., 2017. Prevalence of tuberculous lymphadenitis in slaughtered cattle in Eastern Cape, South Africa. *International Journal of Infectious Diseases*, *61*, pp.27-37.

Grobler, D.G., De Klerk, L.M., Bengis, R.G. and Michel, A.L., 2002. The gamma-interferon test: its usefulness in a bovine tuberculosis survey in African buffaloes (Syncerus caffer) in the Kruger National Park.

Hlokwe, T.M., Jenkins, A.O., Venter, E.H., Michel, A.L., Streicher, E.M., Cooper, D. and Godfroid, J., 2011. Molecular characterisation of Mycobacterium bovis isolated from African

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buffaloes (Syncerus caffer) in Hluhluwe-iMfolozi park in KwaZulu-Natal, South Africa. *Onderstepoort Journal of Veterinary Research*, *78*(1), pp.1-6.

Humblet, M.F., Boschiroli, M.L. and Saegerman, C., 2009. Classification of worldwide bovine tuberculosis risk factors in cattle: a stratified approach. *Veterinary research*, *40*(5), pp.1-24.

Marange, R., Morar-Leather, D. and Fasina, F.O., 2020. Survey of the knowledge, attitude and perceptions on bovine tuberculosis in Mnisi community, Mpumalanga, South Africa. *Onderstepoort Journal of Veterinary Research*, *87*(1), pp.1-4.

Michel, A.L., Cooper, D., Jooste, J., De Klerk, L.M. and Jolles, A., 2011. Approaches towards optimising the gamma interferon assay for diagnosing *Mycobacterium bovis* infection in African buffalo (Syncerus caffer). *Preventive veterinary medicine*, *98*(2-3), pp.142-151.

Raath, J.P., Bengis, R.G., Bush, M., Huchzermeyer, H., Keet, D.F., Kernes, D.J., Kriek, N.P.J. and Michel, A., 1995. Diagnosis of tuberculosis due to *Mycobacterium bovis* in the African buffalo (*Syncerus caffer*) in the Kruger National Park. *Tuberculosis in wildlife and domestic animals, ed. Griffin F, de Lisle G*, pp.313-315.

Sichewo, P.R., Etter, E.M.C. and Michel, A.L., 2019. Prevalence of *Mycobacterium bovis* infection in traditionally managed cattle at the wildlife-livestock interface in South Africa in the absence of control measures. *Veterinary research communications*, *43*(3), pp.155-164.

Sichewo, P.R., Hlokwe, T.M., Etter, E. and Michel, A.L., 2020. Tracing cross species transmission of *Mycobacterium bovis* at the wildlife/livestock interface in South Africa. *BMC microbiology*, *20*(1), pp.1-9.

Wood, P.R. and Jones, S.L., 2000. BOVIGAM: an in vitro cellular diagnostic test for bovine tuberculosis, Tuberc. Edinb. Scotl. 81 (1e2) (2001) 147e155.

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10. Appendices

5.1. Human questionnaire

Section 1: Background information of the individual

1.1 Address of the Abattoir/GPS

1.2 Name of respondent (will be kept confidential)

1.3 Sex of respondent 1. Male ☐ 2. Female ☐ 3. Unknown ☐

1.4 Age of respondent ☐

1.5 Marital status 1. Married ☐ 2. Single ☐ 3. Divorced ☐

1.6 Job description

Butcher/slaughter man ☐

Inspector ☐

Transporter ☐

Others---

1.7 How long have you worked in the abattoir?

One year ☐

Two years ☐

Three years and above ☐

1.8 What animal section do you work in?

Cattle ☐

Sheep/goat ☐

Pig ☐

Games ☐ All sections ☐

1.9 Do you take care of animals at home or work on farms? 1. Yes ☐ 2. No ☐

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1.10 Do you consume unpasteurized milk? 1. Yes ☐ 2. No ☐

1.11 Do you consume uncooked or undercooked meat? 1. Yes ☐ 2. No ☐

1.12 Do you slaughter animals at home? 1. Yes ☐ 2. No ☐

1.13 Do you have personal protective gears? 1. Yes ☐ 2. No ☐

(*If yes*: go to question 3.13.1 *if no* go to question 3.14)

1.14 Do you wear your personal protective gears? 1. Yes ☐ 2. No ☐

1.15 Have you ever had any of the symptoms below in the abattoir? 1. Yes ☐ 2. No ☐

Fever ☐

Cold ☐

Seizures ☐

Back pain ☐

Coughing ☐

Body pain ☐

SECTION 2 :- ZOONOSES

Brucellosis

2.1 Do you know what brucellosis disease is? 1. Yes ☐ 2. No ☐

2.2 Do you think you can get brucellosis from animals abattoir 1. Yes ☐ 2. No ☐

2.3 Do you cook you meat very well before eating? 1. Yes ☐ 2. No ☐

2.4 Have you ever had hand injuries working at the abattoir? 1. Yes ☐ 2. No ☐

Leptospirosis

2.5 Do you know what leptospirosis disease is? 1. Yes ☐ 2. No ☐

2.6 Do you think you can get sick from animals? 1. Yes ☐ 2. No ☐

2.7 Do you see rats around the abattoir? 1. Yes ☐ 2. No ☐

2.8 Do you on other farms apart from the abattoir? 1. Yes ☐ 2. No ☐

2.9 Do you think you can get sick from the abattoir? 1. Yes ☐ 2. No ☐

2.10 Do you use water bodies around the abattoir? 1. Yes ☐ 2. No ☐

Tuberculosis

2.11 Do you know what tuberculosis disease is? 1. Yes ☐ 2. No ☐

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2.12	Have you been vaccinated against TB?	1. Yes	☐	2. No	☐
2.13	Have you ever been sick from tuberculosis?	1. Yes	☐	2. No	☐
2.14	Has any member of your family been sick from TB before?	1. Yes	☐	2. No	☐
2.15	Do you think you can get TB from animals?	1. Yes	☐	2. No	☐

Cysticercosis

2.16	Do you know what cysticercosis disease is?	1. Yes	☐	2. No	☐
2.17	Do you think you can get cysticercosis from animals?	1. Yes	☐	2. No	☐
2.18	Do you defecate in the toilet in your home?	1. Yes	☐	2. No	☐
2.19	Do you defecate in the environment around the abattoir?	1. Yes	☐	2. No	☐
2.20	Have you ever had seizures before?	1. Yes	☐	2. No	☐

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Appendix 5.2 .

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GAUTENG
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

Diamond Building, 11 Diagonal Street, Newtown
PO Box 8769, Johannesburg, 2000
Tel: 011 240 2500
Fax: 011 240 2700

Enquiries : Dr. Wynton Rabolao
Telephone : 011 240 2521
Reference : Consent to Sample in Abattoirs

DEAR ABATTOIR OWNERS,

PERMISSION TO SAMPLE ABATTOIR WORKERS IN SELECTED ABATTOIR IN GAUTENG

Dear Abattoir owner,

The Department (Gauteng Department of Agriculture & Rural Development) in collaboration with the University of Pretoria, is hereby conducting a research on the prevalence and characteristics of selected pathogens (bacterial, protozoan and parasitic) in slaughter livestock and wildlife in abattoirs in Gauteng Province.

This research will be done by interviewing the abattoir managers and workers at the abattoir premise and recording information to assess the risk factors that may predispose to infection. Animals will be sampled and examined before and after slaughter to determine exposure to zoonotic pathogens.

Also samples will be collected by the University of Pretoria students and test for different diseases of zoonotic importance and of public health implication to the workers at the abattoir.

Please kindly grant these student audience as the outcome of this research is to the good of promoting good hygiene practices in the abattoir, as we can further strengthen this point in the abattoirs.

Should you wish to contact us the Department for clarity kindly, please call us on the following numbers: Germiston: 073 735 0932; Pretoria; 083 703 6545 and Randfontein; 071 886 3266

DR. WYNTO RABOLAO (082 373 7724)
DIRECTOR: VPH & Export Facilitation
DATE: 2016/07/22

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agriculture,
forestry & fisheries
Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Forestry and Fisheries
Private Bag X138, Pretoria 0001
Enquiries: Mr Herry Gololo • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HerryG@daff.gov.za
Reference: 12/11/1/1/6

Dr Henriette van Heerden
Department of Veterinary Tropical Diseases
University of Pretoria
Tel: 012 529 8265
E-mail: henriette.vanheerden@up.ac.za

RE: PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO. 35 OF 1984)

Dear Dr van Heerden,

Your application sent with the email on 15 July 2016 requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers.

I am pleased to inform you that permission is hereby granted to perform the following study. *"Prevalence and characterisation of selected pathogens in slaughter animals at Gauteng Province abattoirs – Food safety implications for meat consumers and zoonotic risks posed to abattoir workers"* with the following conditions:

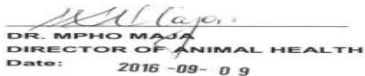
Conditions:

1. This permission does not relieve the researcher of any responsibility which may be placed on him by any other act of the Republic of South Africa;
2. The study must be conducted in compliance with the Veterinary and Para-Veterinary Professions Act 1982 (Act No. 19 of 82) and the Meat Safety Act 2000 (Act No. 40 of 2000);

3. This section 20 approval covers the relevant sub-sections of the main research proposal as declared by the researcher, and does not extend beyond the scope of the specified protocol;
4. Samples must be packaged and transported in accordance the National Road Traffic Act, 1996 (Act No. 93 of 1996);
5. Any incidence or suspected incidence of a controlled or notifiable disease in terms of the Animal Diseases Act 1984 (Act no 35 of 84), must be reported immediately to the State Veterinarian of the area.
6. All potentially infectious material utilised or generated during or by the study is to be destroyed at completion of the study;
7. Only a registered waste disposal company may be used for the removal of waste generated by or during the study;
8. Records must be kept for five years for audit purposes.
9. No part of this study may commence until valid ethical approval has been obtained in writing from the relevant authority;
10. A dispensation for the storage of serum, bacterial isolates and extracted DNA is attached.

Title of research/study: *"Prevalence and characterisation of selected pathogens in slaughter animals at Gauteng Province abattoirs – Food safety implications for meat consumers and zoonotic risks posed to abattoir workers"*
Researcher: Dr Henriette van Heerden
Institution: Department of Veterinary Tropical Diseases, University of Pretoria
Our ref Number: 12/11/1/1/6
Your ref: FY 2015/2016

Kind regards,



DR. MPHO MAJA
DIRECTOR OF ANIMAL HEALTH
Date: 2016 -09- 09

agriculture,
forestry & fisheries
Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Forestry and Fisheries
Private Bag X138, Pretoria 0001
Enquiries: Mr Herry Gololo • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HerryG@daff.gov.za
Reference: 12/11/1/1/6

Dr Henriette van Heerden
Department of Veterinary Tropical Diseases
University of Pretoria
Tel: 012 529 8265
E-mail: henriette.vanheerden@up.ac.za

RE: DISPENSATION ON SECTION 20 APPROVAL IN TERMS OF THE ANIMAL DISEASES ACT, 1984 (ACT NO 35 OF 1984) FOR: "PREVALENCE AND CHARACTERISATION OF SELECTED PATHOGENS IN SLAUGHTER ANIMALS AT GAUTENG PROVINCE ABATTOIRS – FOOD SAFETY IMPLICATIONS FOR MEAT CONSUMERS AND ZOONOTIC RISKS POSED TO ABATTOIR WORKERS"

A dispensation is hereby granted on Point 10 of the Section 20 approval that was issued for the above mentioned study (attached):

i) Serum samples collected from the respective slaughter animals at the specified abattoirs may be stored in the relevant ARC-OVI and UP serum banks;
ii) Bacterial isolates and extracted DNA obtained from the collected samples may be stored in the relevant ARC-OVI and UP serum banks;
iii) Stored samples may not be outsourced or used for further research without prior written approval from DAFF.

Kind regards,

DR. MPHO MAJA
DIRECTOR: ANIMAL HEALTH
Date: 2016 -09- 09

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Appendix 5.3 (approval by ARC-AEC)

AEC 12.-16.

ONDERSTEPOORT VETERINARY INSTITUTE
ANIMAL ETHICS COMMITTEE

Application for clearance to use vertebrate animals (including their embryos and foetuses) for an experimental study or in a standard operating procedure for diagnostic purposes

Where clearance is sought for a standard operating procedure (SOP), a separate clearance form must be completed per procedure

NOTE:

- Please read the Animal Ethics Categories (AEC 01) form before you complete this application.
- This application must be typed.
- It must be signed by the Principal Investigator (the applicant) and other persons who are vouching for specialised aspects of the experimental design (i.e. statistician, safety officer, and persons responsible for supervising the use of scheduled medicinal substances).
- The application needs to be written simply but include all relevant detail.
- A score sheet must accompany all applications.
- If any animal during the experimental period gets sick/dies due to causes not related to the experimental work, a morbidity/mortality report must be submitted to the AEC accompanied by a full post mortem report.
- Once the experimental work starts a copy of the score sheet and a summary of the experimental work conducted must be visible at the experimental facilities in case of inspections by the AEC.
- **Submitting applications:** An electronic copy of the application should be emailed to SmitCH@arc.agric.za Ms Cecilia Smit, Secretary of the Onderstepoort Veterinary Institute – Animal Ethics Committee (OVI-AEC), (Tel: 012-5299378).
- **Deadline** for researchers to submit protocols will be the **1st of each month**. Late protocols go through to the next month.
- An AEC-reference no will be allocated to the submission and it will be distributed electronically to all AEC members for review and comments. Comments from AEC members will be submitted to Ms Smit by the 10th of the month.
- Ms Smit will collate all the comments and send them back to the AEC members, and applicants after the monthly AEC meeting.
- **Approved applications:** the original document with all the relevant signatures must be submitted to the secretary to arranged that it be Stamped and signed by the AEC-Chairman. The original document will be filed on AEC-Submission file and a copy be sent to the applicant.
- Revised applications must be submitted by the 20th of the month.
- AEC meetings will be held on the third Thursday of each month (except December) where all protocols and comments will be discussed, and final committee approval (or not) given, and communicated to applicants asap.
- Telephone enquiries on any animal ethics related matters may be directed to the Chairperson, Dr Paidamwoyo Mutowembwa (Tel: 012-529 9593, or MutowembwaP@arc.agric.za)
- **NB: No animals will be allowed at the ARC-OVI campus unless Section 20 approval is obtained from the Department of Agriculture, Forestry and Fisheries.**

APPLICATION FOR CLEARANCE AEC 2

APPROVED

Submission Date		For Administrative Purposes	AEC REF	AEC 12-16
AEC approval Date	17 August 2016.		Signature (only on approval)	[signature]

A. PROJECT NO:

B. PROJECT TITLE

Prevalence and characterization of selected pathogens in slaughter animals in Gauteng Province abattoirs: Food safety implications for meat consumers and zoonotic hazards posed to abattoir workers

C. PROJECT LEADER/RESEARCHER (PRINCIPAL INVESTIGATOR)

Name	Contact Number		e-mail address	Contact Address
Andrew Potts	0125299396		PottsA@arc.agric.za	P/Bag X5, Onderstepoort, 0110
Division		Diagnosis of Bacterial Diseases		
Qualifications		BSc Hons, PhD		
Appropriate experience in animal research		25		
Details of involvement		Project coordination, leptospirosis research		

D. RESEARCH TEAM MANAGER (RTM)

Name	Contact Number	e-mail address	Contact Address
Claude Sabeta	0125299439	SabetaC@arc.agric.za	P/Bag X5, Onderstepoort, 0110
Division	Food Feed and Veterinary Public Health		
Qualifications	PhD (Molecular Virology)		
Appropriate experience in animal research	At least 20 years (small animals)		

D: Co-WORKERS (involved directly with procedures on Animals

Name	Contact Number	e-mail address	Contact Address
Francis Kolo	0844559876	kolofrancis@hotmail.com	P/Bag X4, Onderstepoort, 0110
Division	University of Pretoria		
Qualifications	DVM, MSc		
Appropriate experience in animal research	14 years experience using experimental animals for diagnostic purposes, for clinical evaluation and treatment.		
Name	Contact Number	e-mail address	Contact Address
Dogonyaro Banenat Bajehson	0125298269	bbdogonyaro@gmail.com	P/Bag X4, Onderstepoort, 0110
Division	University of Pretoria		
Qualifications	MSc		
Appropriate experience in animal research	Experience in sample collection for diagnostic and research purposes.		

- 2 -

Name	Contact Number	e-mail address	Contact Address
Nomsa Mabogoane	0123826398	LetsoaloNF@tut.ac.za	P/Bag X4, Onderstepoort, 0110
Division	University of Pretoria		
Qualifications	BSc Hons		
Appropriate experience in animal research	8 years' experience in animal diagnostics and validation of tests.		
Name	Contact Number	e-mail address	Contact Address
Vuyokazi Mareledwane		MareledwaneV@arc.agric.za	P/Bag X5, Onderstepoort, 0110
Division			
Qualifications	MSc		
Appropriate experience in animal research			

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5.4 Protocol approval by University of Pretoria

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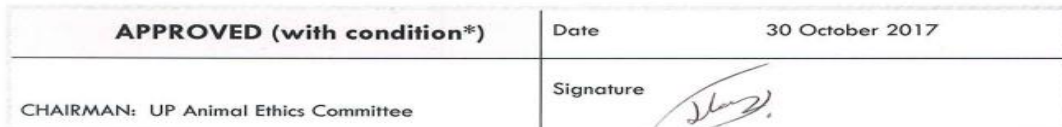
Animal Ethics Committee

PROJECT TITLE	**Prevalence and characterization of Mycobacterium species in slaughter animals in Gauteng province abattoirs: food safety implications for meat consumers and zoonotic risk posed to abattoir workers**
PROJECT NUMBER	**V104-17**
RESEARCHER/PRINCIPAL INVESTIGATOR	**V Mareledwane**

STUDENT NUMBER (where applicable)	**U_25540590**
DISSERTATION/THESIS SUBMITTED FOR	**PhD**

ANIMAL SPECIES	**Cattle, pigs, goats, sheep, game**
NUMBER OF SAMPLES	**500**
Approval period to use animals for research/testing purposes	**October 2017 – October 2018**
SUPERVISOR	**Prof. A Adesiyun**



**CONDITION: Sampling is only approved POST slaughter to minimize stress experienced by animal ante-mortem*

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED (with condition*)	Date 30 October 2017
CHAIRMAN: UP Animal Ethics Committee	Signature

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Faculty of Veterinary Science
Animal Ethics Committee

7 November 2019

Approval Certificate
Annual Renewal (Extension 1)

AEC Reference No.: V104-17
Title: Prevalence and characterization of Mycobacterium species in slaughter animals in Gauteng abattoirs: food safety implications for meat consumers and zoonotic risk posed to abattoir workers
Researcher: Mrs VE Mareledwane
Student's Supervisor: Prof AA Adesiyun

Dear Mrs VE Mareledwane,

The **Annual Renewal** as supported by documents received between 2019-10-11 and 2019-11-05 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2019-11-05.

Please note the following about your ethics approval:

1. The use of species is approved:

Species	**Number Available**
Cattle, Sheep, Goats, Pigs, Game	500 (at abattoir after slaughter)
Samples Fresh blood, liver, lung, kidney, various lymph nodes	All sampling done post slaughter

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2020-11-07.
3. Please remember to use your protocol number (V104-17) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.
Yours sincerely

Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8483
Fax +27 12 529 8321
Email aec@up.ac.za
www.up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

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5.5 Detailed procedural steps of the Bovigam

In the first phase of the test, fresh blood samples were stimulated with purified protein derivatives (PPD). For each animal, blood in the heparin tube was carefully mixed and 1.5 ml aliquoted into 5 individual wells of a 24-well plate. Blood samples were sensitized with 30 µl bovine PPD (600 IU/ml); 60 µl avian PPD (1000 IU/ml) (Prionics AG, Lelystad, The Netherlands); 25 µl PPD-Fortuitum (0.5 mg/ml) in individual wells. As an internal positive control, 11 µl Pokeweed mitogen (5 µg/ml) was aliquoted into the next well of the plate. Unstimulated whole blood from each animal served as negative controls for the assay. The tuberculin and blood were carefully mixed by gentle hand agitation and incubated at 37°C for 20-24 h. After incubation, the samples were centrifuged at 3000 rpm for 10 minutes and 150 µl of the plasma was transferred into appropriately identified tubes with corresponding labels. Plasma samples were stored at -20°C until tested. The plasma samples were assayed for the presence of IFN-γ using a commercially purchased Bovigam® 1G-test kit (Prionics AG, Lelystad, The Netherlands), following the manufacturer's instructions. The production of IFN-γ by the lymphocytes was detected using a monoclonal antibody-based sandwich enzyme immunoassay (EIA). Optical densities were measured on a BioTek ELx800 Plate reader (BioTek Instruments Inc., Winooski, Vermont, USA) at 450 nm. Results were interpreted as previously described (Michel, Cooper, Jooste, de Klerk, & Jolles, 2011).

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