Questionnaire-based study to determine the state of tuberculosis testing in goats in South Africa

by

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DECLARATION

I, Godfrey Nyoni, hereby declare that the research presented in this dissertation, neither the substance, nor any part of this dissertation has been submitted in the past or is to be submitted for a degree at this University or any other University. This dissertation is presented in partial fulfilment of the requirements for the degree MSc Tropical Animal Health. I hereby grant the University of Pretoria free license to reproduce this dissertation in part or as whole, for the purpose of research or continuing education.

Signed

Godfrey Nyoni

Date

June 2019

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LIST OF ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
APHA	Animal and Plant Health Agency
aPPD	Avian Purified Protein Derivative
BCG	Bacillus Calmette–Guérin
bPPD	Bovine Purified Protein Derivative
bTB	Bovine Tuberculosis
CIDT	Comparative Intradermal Skin Test
CMI	Cell Mediated Immunity
DAFF	Department of Agriculture, Forestry and Fisheries
DNA	Deoxyribonucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
HI	Humoral Immunity
HIV	Human Immunodeficiency Virus
IFN-γ	Interferon-gamma
IDT	Intradermal Skin Test
MIRU	Mycobacterial Interspersed Repetitive Unit
MBTC	Mycobacterial Tuberculosis complex
NTM	Non-Tuberculosis Mycobacteria
OIE	Office International des Epizooties
PCR	Polymerase Chain Reaction
PPD	Purified Protein Derivative
rRNA	Ribosomal Ribonucleic Acid
RSA	Republic of South Africa
SICCT	Single Intradermal Cervical Comparative Test
TB	Tuberculosis
UK	United Kingdom
USA	United States of America
VNTR	Variable Number Tandem Repeat
WHO	World Health Organisation

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SUMMARY

Questionnaire-based study to determine the state of tuberculosis testing in goats in South Africa

BACKGROUND

In South Africa, bovine TB (bTB) is a state-controlled disease but the monitoring of bTB in small ruminants is largely neglected, whereas bTB diagnosis and surveillance in cattle and buffalo are prioritised due to limited resources. In communities where bTB surveillance is not conducted regularly in herds, bTB surveillance data in cattle and other susceptible livestock are lacking. Goats co-existing with cattle, especially in areas where the prevalence of TB is high in both the cattle and the human populations, are not tested for TB. The prevalence of bTB in goats is unknown though they are known to be susceptible to *Mycobacterium bovis*. Thus, there was an opportunity to gather information from state veterinarians on their observations of bTB in goats. This information would assist to make a decision on whether to pursue further studies on bTB in goats in South Africa.

METHODS

A survey study design using a quantitative research approach was used. Data were collected through face-to-face interviews using a semi-structured questionnaire.

Contacts of state veterinarians where obtained from the Department of Agriculture Forestry and Fisheries (DAFF) website. Participants were initially contacted via email. In the e-mail the participants were given a brief introduction of the researcher as well as his intention of carrying out the questionnaire.

Participants that were willing to participate would either do so via email in which they would read and sign the consent form and then proceed to answer the questions on the questionnaire. They could either fill in with a pen or, type in their responses electronically.

During the face-to-face interviews, participants were given the option to complete the questionnaire themselves or let the interviewer read out the questions and complete the questionnaire on their behalf. They were however requested to go through the consent form and fill it in before the interview could start. Other participants preferred to go through the questionnaire themselves while in the presence of the interviewer.

RESULTS

None of the 25 interviewed veterinarians had tested for or diagnosed bTB in goats. When asked the reason for not testing for bTB in goats, 55% said it was not mandatory and 25% cited limited resources as the reason.

From the literature search, Spain and the United Kingdom had the most reported cases in Europe of TB in goats at 36% (n=355) and 27% (n=45) respectively. *M. bovis* was the most commonly encountered species and the mediastinum lymph nodes was the organ most often affected. In Africa, Ethiopia and Nigeria had the most reported cases of TB in goats with 54% (n=184) and 15.4% (n=9) respectively. *M. bovis* was most commonly encountered in these cases and unlike the European cases; the lungs were most frequently affected with a frequency of just under 80%. The only reported case of bTB in goats from South Africa was reported in 1928.

CONCLUSION

There is limited knowledge of bTB in goats in South Africa primarily driven by a bTB disease policy that is biased to focus on cattle and pay little attention to small stock such as goats.

Chapter 1

Introduction

1.1 Background

1.1.1 Goats

Goats are hardy, inquisitive and intelligent animals and can be found in every community on the African continent (Harwood, 2014) (Peacock, 2005). Zulu and Xhosa households in rural South African communities use their goats mostly for meat, milk and skin production while Venda, Tsonga and Pedi tribes use goat milk for their household requirements (Gundidza et al., 2008). Goats are not only kept for their milk, meat and skin, but goats can be liquidated to generate cash to pay for things such as tuition. (Gwaze et al., 2009). In addition, goats play an important role in the traditional and cultural practices such as payment for brides, important rituals and rites, and are sacrificed for important visitors (Lebbie, 2004). Although goats present many valuable attributes, they are sometimes associated with poverty because of farmer ignorance (Peacock, 2005).

The majority of South Africa's 6 million goats fall into two broad categories that is, the commercial farmers and the small-scale farmer. An example of a breed of goat the commercial sector focuses on is the Angora. It is mainly kept for mohair production with surplus animals sent for slaughter. There is sufficient goat reserves in the country, predominantly owned by the non-commercial farmers, to supply a regular supply to market. (Roets and Kirsten, 2005).

1.1.2 Tuberculosis (TB) in goats

Several wildlife species in South Africa have been found to have tuberculosis (TB) which was attributed to infection with *Mycobacterium bovis* (Lisle et al., 2002). African buffalo (*Syncerus caffer*) is the maintenance host. *M. bovis* infected buffalo not only serve as a source of infection to other wildlife but also to domestic livestock (Musoke et al., 2015), such as communal areas bordering national parks where bovine TB (bTB) is endemic. Among domestic livestock, cattle are thought to be the maintenance host of *M. bovis*. However, TB has been reported in a number of other livestock species such as pigs, sheep and goats (Huitema, 1972). These species are susceptible to infection by both *M. bovis* and *M. tuberculosis*, the pathogen that causes TB in humans (Cosivi et al., 1995, Cousins, 2001).

In South Africa communal farmers own cattle, but many may also own goats. In this type of environment goats may share grazing pastures but also water resources and housing. In some cases owners even share their abodes with their livestock (Lebbie, 2004). These conditions may increase the prevalence of TB in both cattle and in humans, and as such may serve as a source of potential infection to goats or other livestock that are susceptible to *M. bovis/M. tuberculosis*.

Transmission of *Mycobacteria* can occur by several routes; a contributory factor which determines which route is the where the foci of the infection is situated (Cosivi et al., 1995). The airborne route appears to be the main route, which facilitates transmission of *M. bovis* among both wildlife and domestic species. Transmission is more likely to occur when infected and non-infected animals are in close proximity to one another. (Morris et al., 1994). Spread of the infection within the environment is facilitated via the dissemination of semen, faeces, urine and other bodily excretions. (Cosivi et al., 1995).

To date very little or no data is available on the prevalence of TB in goats in South Africa. Goats are regarded as spill-over hosts of *M. bovis* (Liebana et al., 1998) (Daniel et al., 2009). However, if the environment is conducive and the animals are found in larger densities they may be able to sustain disease and thus serve a as a source of infection to cattle, other livestock and to their owners, who may consume unpasteurised goat milk or *M. bovis* infected goat meat (Higino et al., 2011).

According to the Department of Agriculture, Forestry and Fisheries (DAFF), the first time TB was recorded in cattle in South Africa was in 1880. (Department of Agriculture, 2016), and it is generally believed that the pathogen was introduced by diseased cattle from Europe during the European settlement in Africa. In 1902, the disease had not been reported in the Natal but was eventually found in large numbers of imported cattle. These animals were deposed of. Despite these efforts the disease still found its way and spread throughout the country affecting mainly the dairy herds. The disease eventually found its way into beef production as the farming systems changed to a more intensive style. Due to the increasing prevalence, TB was declared a notifiable disease in 1911 by the Veterinary Services of South Africa (Department of Agriculture, 2016). Since then, various programmes have been introduced to control the disease; the present one was introduced in 1965.

Bovine TB is one of many controlled diseases in South Africa as prescribed by the Animal Diseases Act, 1984 (Act 35 of 1984) and the Standing Regulations enacted in terms of this Act. The Bovine Tuberculosis Eradication Scheme was put in place to eradicate bTB from South Africa. Following the implementation of test and slaughter campaigns, the prevalence of bTB dropped 1991. Since then testing has greatly declined. The decline is attributed to several factors including shift in farming practices and constraints on the budget. Superficially, the communal farming regions enjoy a low prevalence of TB. In addition, it would seem that

Zimbabwe, Namibia and eSwatini (formally known as Swaziland) all have a very low prevalence of TB in their communal cattle (http://hdl.handle.net/2263/36811).

Tuberculosis is a difficult disease to diagnose in goats, as they appear clinically healthy and do not present with any clinical signs until disease is advanced (Schwabacher, 1934). Infected goats may show gradual loss of body condition, develop respiratory problems and start coughing. Other signs include occasional diarrhoea, generalised weight loss, occasional enlarged lymph nodes and in females there is usually a drop in milk production (Quintas et al., 2010) (Liebana et al., 1998) (Crawshaw et al., 2008).

Detecting infected animals and removing those animals that are shedding the pathogen, are important measures that can be taken towards TB eradication programmes. Diagnostic assays aimed at cell mediated immunity (CMI) and humoral immunity (HI) responses such as the intradermal test (IDT), Bovigam® (IFN-y assay) and antibody ELISAs can be used to diagnose bTB in goats (Bezos et al., 2015). Molecular-based assays such as PCR can be used to determine the cause of the TB in goats as well, since goats are susceptible to most of the pathogens in the *Mycobacterium* TB complex (MTBC). To confirm TB, bacteriological culture remains the gold standard (Gutiérrez et al., 1998) (Zanardi et al., 2013).

1.2 JUSTIFICATION

South Africa's goat population makes approximately 3% of Africa's goats and less than 1% of the global goat population. For the past 10 years (2004-2014), the average gross value of chevon (goat meat) has amounted to R378 million per year. Although found throughout the country, Eastern Cape, Limpopo and KwaZulu-Natal provinces are the largest producers making up 71% of the total live goats. Exports of chevon from South Africa totalled 54 152 kilograms valued at R3 134 237 during the past decade (2004-2014). South Africa exports chevon to Gabon (20%), Qatar (17%), Mozambique (10%), Tanzania (1%), Ethiopia (11%), DRC (17%), Seychelles (23%) and Saint Helena (1%) (Department of Agriculture, 2014).

Guidelines on the standards of testing for TB have been released by the Office International des Epizooties/World Organization for Animal Health (OIE). The guidelines released have been with respect to cattle and to date no guidelines applicable in small ruminants have been released. There has been an increase in reports of small ruminants being infected by *M. bovis* in Europe (Tschopp et al., 2011).

Despite the low prevalence of TB in goats, Spain has included goats into testing and slaughter programs. Testing is compulsory for mixed herds or in situations where goats share pasture with cattle.

Mycobacterium caprae (Aranza et al. 2003), causing TB in goats, was isolated from a herd of cattle in Spain. The goat herd implicated as the source of the infection were housed 100 m from the cattle. The goat herd consisted of 254 animals and upon single intradermal comparative cervical tuberculin (SICCT) test 79.5% (n=202) of the goats were skin test positive for caprine TB and the causative agent was isolated from 10 goats (Napp et al., 2013). Subclinical TB infections in goats was a contributor of persistent infection in cattle in conditions where cattle and goats shared pasture or were in close contact (Blasio et al., 2017) .Though the infection is subclinical in goats, the presence of the pathogen leads to environmental contamination and has a negative impact on eradication programs.

In Italy surveillance of TB in goats is not mandatory except in raw milk producers. This approach to disease surveillance leads to an information gap as it focuses only on goat milk producers and not on other goat production systems that can also be affected by bTB. In parts of Spain where you find a high incidence of bTB, commercial goat herds are regularly included in the TB eradication programme (Acosta et al., 1998).

In South Africa, the government follows a "slaughtering out" policy for infected cattle under the Tuberculosis Eradication Scheme. The purpose of this scheme is the total eradication of the disease in South Africa (Department of Agriculture, 2016). According to the Tuberculosis Scheme Manual, "*The natural resistance of goats is not high and where they are exposed to a high degree of infection with M. bovis large numbers can be infected. Where infection in cattle is determined it will be necessary to pay attention to goats especially if there is close contact between them and cattle. The infection causes a disease that develops slowly in goats but may be the cause for the disease spreading to other animals.*" With this in mind, the same manual states that the scope of the bovine TB eradication program is limited to *M. bovis* in cattle (Department of Agriculture, 2016). Despite this being the official stance of DAFF with respect to TB, it would be interesting to know the opinions of states veterinarians with respect to TB in the field and comparing it to government policy.

1.3 PROBLEM STATEMENT

In South Africa, bTB is a state-controlled disease but the monitoring of TB in small ruminants is largely neglected, and TB diagnosis and surveillance in cattle and buffalo are prioritised in view of limited resources and the economic incentive of having TB free cattle.

In rural communities in South Africa, families own cattle, but also other livestock. In communities where TB surveillance is not done regularly in herds, bTB surveillance data in cattle and other susceptible livestock are lacking. Goats co-existing with cattle, more importantly in areas where the prevalence of TB is high in both the cattle and the human populations, are not tested for TB. The prevalence of TB in goats is not known though they are known to be susceptible to *M. bovis*. However, in view of the zoonotic potential and public health risks, this needs to be given more attention.

Thus, there is a need to gather information on this topic from state veterinarians on their observations of TB in goats. This information would put us in a better position to decide on future studies on TB in goats in South Africa.

1.4 HYPOTHESIS

Goats are not tested for TB in South Africa

1.5 AIM

The aim of this project is to determine the status of TB testing in goats in South Africa.

1.6 OBJECTIVES

The research objectives of this study were to ascertain the knowledge of state veterinarians about TB testing in goats, to identify the tests used for TB diagnosis in goats and to determine the validation status of TB diagnostic tests used in goats in South Africa.

Chapter 2

LITERATURE REVIEW

2.1 TUBERCULOSIS

Tuberculosis affects wildlife, livestock and humans. It can impact many organ systems depending on how it enters the host. It is a disease that is chronic in nature and is caused by members of the *Mycobacterium tuberculosis* complex (MTBC) which comprise of *M. africanum M. bovis, M. canettii M. caprae, M. microti, M. pinnipedii and M. tuberculosis.* All mycobacteria are acid fast and can affect most vertebrates. Other newer and lesser known members of the MTBC include *M. mungi*, *M. orygis*, *M. suricattae* and the Bacillus Calmette-Guerin strain of *M.bovis*. https://en.wikipedia.org/wiki/Mycobacterium_tuberculosis_complex

M. tuberculosis has been found to be the leading source of TB in humans while *M. bovis* is the dominant cause of TB in animals and is also responsible for zoonotic TB (Vayr et al., 2018). As *M. bovis* has been found to be ubiquitous among domestic animals, consequently, it has been described in great detail in both free-ranging and captive populations of wildlife. Cattle are regarded as the domestic host of this pathogen. Endemically infected animals such as the European badger *(Meles meles)* and the African buffalo play a role in the maintenance of the pathogen within the environment. These wildlife reservoirs hamper TB control efforts and ultimately pose a considerable threat to public health.

Other than being the main human pathogen, *M. tuberculosis* has been found and documented in domestic or wildlife species living in close proximity for extended periods of time, with people (Alexander et al., 2002). Tuberculosis still remains a major global health problem (Organization, 2013).

2.2 MYCOBACTERIA OF THE *MYCOBACTERIUM TUBERCULOSIS* COMPLEX

Tuberculosis has spread and is now encountered in most parts of the world. Mycobacteria that are responsible for human and animal diseases are grouped together and form what is known as the MTBC (Huard et al., 2006). This complex consists of all the mycobacteria that cause TB in their hosts. Bacteria of MTBC are closely related such that they are generally considered as subspecies and are 99.9% similar at the nucleotide level, with identical 16S rRNA sequences (Brosch et al., 2002). Regardless of the similarities that they share, they do however have distinct differences at the phenotypic level as well as varying host ranges and pathogenicity (Ayele et al., 2004).

In order to allow for a more accurate diagnosis and therefore appropriate remedial action differentiation of MTBC members becomes important and takes on a more critical role as it also has public health implications. In countries such as Zimbabwe, South Africa and Mozambique, there exists only limited information on the total incidence or prevalence of mycobacterial disease due to specific MTBC members (Michel et al., 2009).

2.3 TUBERCULOSIS IN DOMESTIC ANIMALS

Despite cattle being regarded as the primary hosts of *M. bovis*, other domestic animal species that have been affected by *M. bovis* include pigs, sheep and goats.

2.3.1 CATTLE

ETIOLOGICAL AGENT

The main cause of TB in cattle is *M. bovis* (Cousins, 2001). TB in cattle caused by *M. bovis* is a disease of note in animals and has zoonotic potential. Infection attributed to *M. tuberculosis* has been seen in a variety of domestic animals which often, are in close contact with people. The domestic animal mostly associated with *M. tuberculosis* infection are cattle (Ocepek et al., 2005).

TRANSMISSION

Cattle are the primary domestic hosts of *M. bovis.* Infectious cattle can shed *M. bovis* in a number of ways such as, in faeces, urine, saliva as well as from milk and discharging lesions (Neill et al., 1991). Husbandry methods also influence the spread of disease. Intensive livestock farming practices places animals into proximity with each other facilitating the spread of *M. bovis*. Close contact between animals can occur at water points such as ponds, wells and streams. Other centres of high animal concentration are vaccination and artificial insemination centres, dipping tanks, auction stations, market places and transportation are the common animal gathering places, and again are sites where transmission of infection could easily occur (Ayele et al., 2004) .

Housed cattle are infected mainly by inhaling infected droplets. Another route of infection is through ingestion and this can take place while grazing on infected pasture and contaminated water sources. Young animals typically get infected when they consume milk that in contaminated by *Mycobacteria.* Infection via this route typically occurs in the late stages of infection. (Radostits et al., 2006).

CLINICAL SIGNS

Affected animals present with pulmonary signs represented by a chronic cough as a result of bronchopneumonia. The cough is rarely loud or continuous, usually suppressed and taking place once or twice at a time, moist and low in tone. As the disease progresses, it results in damage to the lung resulting in dyspnoea affecting both the depth and rate of respiration negatively. Enlargement of the retropharyngeal lymph node results in difficulty in swallowing, as well as loud breathing due to obstruction around the pharynx. Palpation of the pharynx reveals a firm, large, rounded swelling in the roof of the pharynx (Radostits et al., 2006).

PATHOLOGY

The disease is characterised by the presence of tuberculous granulomas. These can occur in several or all the lymph nodes but have been primarily found in mediastinal, bronchial and retropharyngeal lymph nodes. The extension of miliary abscesses throughout the lung results in a suppurative bronchopneumonia. The pus generated as a result of this is characterised by a cream to orange colour and its consistency varies from thick cream to crumbly cheese. Apart from the above mentioned locations, tuberculous nodules may be seen on the pleura and in the peritoneum (Radostits et al., 2006).

2.3.2 SHEEP

M. bovis infections are rare in sheep, most likely due to the husbandry practices that sheep are exposed to as well as their nature. These two factors result in a reduced exposure to potential material of an infectious nature (Cousins, 2001). The behaviour of sheep and the management practice they are exposed to influences the occurrence of TB in sheep. Sheep are not usually intensely managed and tend to flock together reducing their contact with potential cattle carriers. Sheep are more cautious animals compared to goats, exhibiting allelomimetic behaviour. Furthermore, sheep graze during the day, thus reducing potential contact with cattle or wildlife that may be infected with *M. bovis* (Broughan et al., 2013).

Another possible reason that could explain the low incidence of TB reports in sheep is that small ruminant carcasses usually go through a less thorough post-mortem examination as compared to cattle. This could be why TB is underdiagnosed in sheep .(Pesciaroli et al., 2014) (Adem and Agga, 2011). *M. bovis* causes lesions in sheep similar to those in cattle. Lesions in sheep have been seen in the lungs, bronchial and mediastinal lymph nodes as well as the kidneys. *M. avium* also affects sheep resulting in pulmonary lesions. The intradermal skin test (IDT) has been found to be useful in diagnosing TB in sheep (Thoen et al., 2009). Due to the fact that sheep develop lesions that affect the respiratory system, they can be included as potential disseminators of infection (Broughan et al., 2013).

2.3.3 PIGS

Pigs can be easily infected by *M. tuberculosis*, *M. bovis and M. avium sp hominisuis*. *M. tuberculosis* outbreaks are known to occur in pigs and tend to be associated with feeding of unprocessed waste from hospitals and sanatoriums. *M. bovis* outbreaks tend to occur in facilities where TB has been recently diagnosed in cattle (Thoen et al., 2009). According to Cousin et al., (2001) ,"the oral route is the most important route of infection in domestic pigs, most frequently caused by consuming milk, milk products or offal from infected cows"(Cousins, 2001).

In Spain, Italy and Portugal, collectively known as the Iberian region where bTB control measures are in effect, infection of swine with *M. bovis* is rare. However, *M. bovis* has been reported in a few enzootic locations. In these situations cattle have been singled out as the source of infection with transmission occurring via the respiratory route. (Parra et al., 2003).

Most lesions in the pig can be in the mandibular and the mesenteric lymph nodes. An extension of infection from these organs may produce lesions in the lungs, spleen and liver. The lesions are typically fibrotic and may, on occasion, be calcified as well as caseous. The subsequent decline in prevalence of *M. bovis* following the removal of the source of infection has supported the suggestion that pigs are end hosts for *M. bovis* (Parra et al., 2003). There is little to no evidence that point to the possibility of pig to pig transmission occurring (Thoen et al., 2009).

2.3.4 GOATS

ETIOLOGICAL AGENT

It was previously believed that goats had an inherent natural resistance to TB; but it has been concluded that infection with *M. bovis* and *M. caprae* can result in TB disease in goats (Álvarez et al., 2008). Tuberculous like disease can also be produced by infection with *M. tuberculosis* and NTM respectively, and reduce the accuracy of the TB diagnostic tests as demonstrated for *M. avium* subsp. *paratuberculosis* (Cadmus et al., 2009) (Gezahegne Mamo et al., 2012) (Tschopp et al., 2011).

Clinical signs

Infected goats appear clinically healthy making diagnosis of TB based on clinical signs during the early stages of infection difficult. Clinical signs usually appear when the disease is at an advanced stage (Schwabacher, 1934) (Bernabé et al., 1990) (Daniel et al., 2009) (Harwood, 2014). Clinically, TB in goats shows the hallmarks of bronchopneumonia overtly shown by cough and terminal dyspnoea (Radostits et al., 2006). Goats that are affected often exhibit a gradual loss of muscle mass, develop respiratory complications and start coughing. Other signs associated with the disease process in goats include periodic diarrhoea, generalised weight loss, occasional enlarged lymph nodes and in females there is usually a reduction in milk production (Bernabé et al., 1990) (Liebana et al., 1998) (Crawshaw et al., 2008, Quintas et al., 2010).

Pathology

Post-mortem findings in goats may vary but usually include the presence of nodules in lungs and lesions of differing sizes containing caseous material highly suggestive of *M. bovis* infections. Often there are fluid filled cavities and extensive tuberculous in the lung tissue. Depending on the route of infection, lesions are found in the mesenteric retropharyngeal, thoracic, and mediastinal lymph nodes, liver, spleen and udder (Schwabacher, 1934) (Bernabé et al., 1990) (Daniel et al., 2009) (Quintas et al., 2010) (Pesciaroli et al., 2014). Other than the above mentioned post-mortem picture, intestinal ulcerations with loose stool and enlargement of the lymph nodes of the alimentary tract may be seen (Radostits et al., 2006).

Epidemiology

Epidemiological studies indicate that TB in goats has a global distribution (Pesciaroli et al., 2014). TB in goats has been diagnosed in France, USA, Great Britain, Italy, Germany, Africa, India, Australia and New Zealand (Wayne and Diaz, 1986). According to an OIE report (OIE, 2008) TB in goats in Africa has been observed in abattoirs from the Ivory Coast (Carmichael, 1939). In Ethiopia, studies conducted in different abattoirs (Adem and Agga, 2011) (Nigussie, 2005) that were supported by mycobacterial culture isolation, showed occurrence of TB in goats (Tafess et al., 2011)

In most cases, infection takes place following contact between infected cattle and susceptible goats. In an outbreak of caprine TB that occurred in India between 1941 and 1942, it was found that the goats had been sharing pasture with heavily infected cattle. The goats had been grazing with a cattle herd that had a TB prevalence of 35%. Despite the high prevalence and the abnormally high stocking density, only one goat was found to have been infected. The goat in question was not overtly malnourished and was only diagnosed after skin testing and subsequent culture of lymph nodes (Lall, 1969). A low prevalence of TB in goats has also been reported in Taipei, China.

The presence of TB in Mediterranean countries such as Spain, Italy and Portugal appears to be widespread and has caused concern in some locations. Mixed farming of cattle and goats is not commonly practiced in these regions but grazing on the same pasture affords close contact between cattle and goats. Until recently it was widely accepted that TB in goats in Spain was cause by infection from *M. bovis*. However, following the application of DNA fingerprinting techniques, strain differences were identified leading to the discovery of *M. caprae* (Cousins, 2001).

RISK FACTORS (TO OTHER ANIMALS)

ENVIRONMENT RISK FACTORS

Husbandry practices, such as animal housing, high stocking intensity and large number of animals on a farm can predispose to disease occurrence. The transmission of TB is likely to occur where infected animals are in close proximity with susceptible healthy animals. (Radostits et al., 2006). However, close contact between carriers and susceptible animals may not necessarily be a prerequisite for disease transmission. In an incident described by Napp et al., (2013), goats were found to have been the source of an outbreak of *M. caprae* among cattle. The cattle and goats did not share pasture nor were they on the same farm. These carrier goats where some distance away on another holding and infection was thought to have been transferred by contaminated slurry from the infected goat herd (Napp et al., 2013).

In Africa, grazing animals usually gather at night for protection from predators. Due to the high ambient temperature in tropical zones, animals tend to come together in large numbers beneath the shade of trees or other shaded areas for parts of the day, preferring to graze early in the morning and late in the afternoon. Close contact between animals which facilitates ease of spread of disease between animals is favoured by intensive production systems. (Ayele et al., 2004).

PATHOGEN RISK FACTORS FOR MTBC

Mycobacteria are relatively resistant to desiccation, heat and a variety of disinfectants. In conducive conditions of warmth, moisture and protection, the organism may be viable for weeks. (Radostits et al., 2006).

Zoonotic TB

Zoonotic TB is important from a public health perspective the world over and more so in developing countries. This is due to deficiencies that exist in African nations particularly in public health and disease control, coupled with a high prevalence and incidence of Human immunodeficiency virus (HIV) in the human population (Etter et al., 2006). Immunocompromised people who may be undergoing chemotherapy or infected with HIV, are particularly prone to infection by *Mycobacteria* that usually infect animals such as *M. bovis* and *M. caprae* (Ayele et al., 2004). The severe impact of HIV disease on human TB owed to *M. tuberculosis* has been well documented. The likelihood of an HIV-TB co-infected individual to develop active TB increases from less than 1% to 8 – 10% annually, and such disease often progresses early in the course of the HIV infection (Grange et al., 1994). Given the rapidly growing worldwide HIV pandemic in emerging countries, the World Health Organisation (WHO) estimates that 70% (6 million) of humans jointly infected with TB and HIV reside in sub-Saharan Africa (Ayele et al., 2004).

Bernabé and colleagues reported that goats provide little defence to both bovine and bird type tubercle bacilli (Bernabé et al., 1990). This is of significance to the immune compromised individuals that stay in a rural areas where access to pasteurised milk is a challenge, making them more susceptible to contracting TB. People can become infected via the consumption of unprocessed animal by-products or by close proximity to infected animals such as livestock (Tschopp et al., 2011).

It is stated in the South African constitution as well as the Meat Safety Act 40 of 2000 that, "slaughtering animals for home consumption or religious reasons outside designated areas (abattoirs) is permitted (Meat Safety Act 40, section 7(2) (b)) provided that the meat is not sold to the general public". During traditional or ritual sacrifice of animals, food safety protocols are usually not adhered to; this results in the potential exposure to practitioners and consumers alike to pathogens such as *Bacillus anthracis*, *Brucella bovis* and *M. bovis*. According to South African law, certain diseases must be reported to the authorities once encountered, examples of such diseases include Rift Valley fever, anthrax, rabies and bTB. Currently, the South African Meat Safety Act, has provision for the slaughter of livestock for traditional purposes without the presence or participation of food safety experienced individuals, which is a risk to the public (Qekwana and Oguttu, 2014).

2.4 DIAGNOSIS OF TUBERCULOSIS

Tests used in the diagnosis of TB are broadly divided into post-mortem and ante mortem diagnosis. Diagnosis can include detection of the actual organism using molecular techniques and or culture on growth media (gold standard), the pathology following interaction of the pathogen in the host and also humoral and cell mediated immune responses to infection (Cousins and Florisson, 2005).

Despite the existence of TB testing standards and guidelines for cattle, similar guidelines and standards for small ruminants (goats and sheep) have not been published by the OIE (Tschopp et al., 2011).

Several tests exist that can be utilised to ascertain the immunological response of a host against *M. bovis* infection. One such test is the IDT, which can be the single and/or comparative procedures. Although no guidelines have been put forward by the OIE, these have been occasionally applied to goats and have proved useful. Varying cut-off points have been suggested and there are many ways of interpreting them. Regardless, weaknesses associated with skin testing have been noted. These limitations are mainly associated with alterations in reactivity more specifically a reduction in reactivity following testing. To overcome these limitations of the IDT, other tests have been developed and tested in cattle. These include the IFN-γ assay and ELISA for the detection of specific antibodies (Gutiérrez et al., 1998).

According to the OIE terrestrial manual 2015, TB diagnostics tests can be broadly classified into identification of the agent, delayed hypersensitivity tests and blood-based laboratory tests.

2.4.1 IDENTIFICATION OF THE AGENT

Examination of stained smears from affected tissues or organs can be used to demonstrate the presence of *M. bovis* and its presence can be confirmed by culture on primary isolation medium. (OIE, 2008).

MICROSCOPIC EXAMINATION

Microscopic examination can be applied on smears from clinical samples or on prepared tissue material to identify *M. bovis* (OIE, 2008). Suitable clinical samples include coughed up mucus; the sediment remains after a milk sample has been centrifuged and impression smears from lymph nodes; or other excretions and organs (Department of Agriculture, 2016). The Ziehl–Neelsen stain is used to demonstrate the acid fastness of *M. bovis*. An alternative, the fluorescent acid-fast stain, can be used as well for the detection of *M. bovis*. A diagnosis of mycobacteriosis can be presumptively made based on the appearance of tissue. A diagnosis on presumptive grounds can be made based on a set of characteristic histological changes. These lesions include macrophages, mineralisation, and epithelioid cells. (OIE, 2008).

CULTURE

Bacterial culture is considered the gold standard and therefore the definitive diagnostic test and should be applied on samples from all suspect and positive herds. The disadvantage of culture is the long time it takes for the organisms to grow (Department of Agriculture, 2016). Bacterial culture involves several preparatory steps which involves homogenising, decontaminating and centrifugation of the sample. Before culture can take place, specimens undergo several steps. In the first step, using a mortar and pestle, tissue is homogenised. The next step after homogenisation is decontamination. This can be achieved by using a chemical detergent such as 0.375–0.75% hexadecylpyridinium-chloride [HPC], an alkali (2–4% sodium hydroxide) or an acid (5% oxalic acid). The resultant solution is thoroughly mixed by shaking for 10 – 20 minutes at room temperature and then neutralised. After centrifugation, the supernatant is discarded leaving the sediment which is used for microscopic examination as well as culture. To perform a primary isolation the sediment is also inoculated into solid egg-based media, such as Lowenstein–Jensen. Cultures are incubated for a minimum of 8 weeks at 37^{o}C. The media surface is evaluated for signs of growth during set intervals throughout the incubation period. After growth is noticeable, stained slides are made using the Ziehl–Neelsen method. A presumptive diagnosis of *M. bovis* can be made on the appearance of colony morphology, however every isolate needs to be confirmed with PCR. Identification of the isolates is based on standard protocols involving molecular tools or traditional cultural and biochemical properties (OIE, 2008).

MOLECULAR TECHNIQUES TO DIAGNOSE TB IN CATTLE

The Gen-Probe AccuProbe culture identification tests (DNA probes) can be used for the quick identification of isolates to the level of MTBC. The test targets the 16S–23S rRNA, the insertion sequences IS6110 and IS1081, and genes coding for MTBC proteins, such as MPB70 and the 38 kDa antigen b. The 38 kDa antigen b of the *M. tuberculosis*H37Rv reference strain, is an immunodominant antigen used in disease diagnosis and in the development of vaccines (Singh et al., 1992). PCR has been applied in the diagnosis of TB in animals but it has shown variable and less than satisfactory results during inter-laboratory comparisons (Noordhoek et al., 1996). PCR generates false positives which may be related to inappropriate testing and lab practice conditions and false negatives related to sub optimal sample processing methods or conditions when applied to samples that contain low bacilli numbers and therefore low copy of target sequences (DNA) per bacilli which ultimately lowers its reliability. The sample may be positive, but the PCR is not sensitive enough to detect the low concentrations of DNA. There are various methods of decontamination, extraction of DNA and techniques for the elimination of polymerase enzyme inhibitors, internal and external controls and procedures for the prevention of cross contamination. All of these variables affect the quality of the PCR process and therefore the quality of result. All of these variables affect the quality of the PCR process and therefore the quality of result (OIE, 2008).

MTBC isolates can be distinguished by a number of DNA-fingerprinting techniques (Durr et al., 2000), which have been developed for epidemiological purposes. These techniques can be used to differentiate different strains of *M. bovis* and will allow patterns of origin, transmission and spread of *M. bovis* to be better described (OIE, 2008). However, according to the DAFF, PCR is not considered a good practical tool and is considered to be an auxiliary test that despite being very sensitive, is also very costly and time consuming compared to other tools such as the interferon-gamma (IFN-γ) assay and IDT and usually restricted to research studies (Department of Agriculture, 2016).

2.4.2 DELAYED HYPERSENSITIVITY TESTS

The IDT is the most frequently used test for the detection of TB. The testing procedures comprise of injections that are intradermal in nature. The substances injected are tuberculin, avian or bovine purified protein derivatives (aPPD/bPPD), which stimulates the development of a swelling (delayed hypersensitivity) at the site of injection 72 hours later in animals that have been exposed to *M. bovis* (OIE, 2008). Before the injection can be administered, the injection site is clipped and sterilised. The injection sites are separated by 12 – 15cm. Where it is not possible to separate the injections sites on the same side of the neck, one injection can be administered on either side of the neck. A short needle is inserted at an angle into the deep layers of the skin. A dose of no less than 2000 international units (UI) of bovine or avian tuberculin is injected. The skin fold thickness s then read after 72 hours. The cut off points for the swellings that develop post injection are read differently for cattle and goats. The OIE has not put forward such cut off points and so different cut off points exist for different situations. In cattle, a negative result is associated with an increase in skin thickness of no more than 2 mm along with the absence of clinical signs. In this case the clinical signs refer to diffuse or extensive oedema, exudation, necrosis, pain or inflammation of the lymphatic ducts in that region or of the lymph nodes. A result is deemed to be inconclusive if the increase in thickness is between 2 mm and 4 mm along with the absence of clinical signs. A positive result is an increase of greater than 4 mm accompanied by the presence of clinical signs. The test in the diagnosis of goats is similar but what differs is the amount of PPD injected as well the cut off points for the size of the swellings.

The IDT determines CMI responses to PPD. False positives occur because the proteins of the PPD may be shared among the different members of the MTBC. To help limit the effect of false positives, the SICCT is used and compares the result of bPPD against result of aPPD (Álvarez et al., 2008). On occasion, the single and comparative tests have been used on goats and have proved to be valuable, resulting in a sensitivity of (92.5%) and specificity of (90%) (Menchén Ozaita, 1995).

In a paper reviewing diagnostic tests for *M. bovis* in non-bovine animals, in which a literature review was conducted and researchers asked for their experiences with the skin tests, the sensitivity and specificity of the IDT was reported to be 100% by 17 references as cited in (Cousins et al., 1993). The CIDT was found to have a sensitivity of 83.7% and specificity of 100%. This evaluation was based on 47 *M. bovis* positive samples and 1 *M. bovis* negative sample (Cousins and Florisson, 2005). In work previously done by Gutiérrez, Tellechea et al, they had found the comparative test to have a sensitivity of 83.7% and a specificity of 100%, (Gutiérrez et al., 1998).

2.4.3 BLOOD BASED LABORATORY TESTS

Other than the IDT, there are several other blood-based tests that have been developed for the diagnosis of TB in goats. These tests are generally more complex and are regarded as ancillary tests used to augment and enhance the more widely IDT test. (OIE 2008). It is reported to increase sensitivity by up to 20% (Department of Agriculture, 2016).

CMI-BASED ASSAYS

In the IFN-γ assay, the measurement of cytokine IFN-γ is performed from whole blood culture. The assay measures the release of IFN-γ from sensitised lymphocytes during a 16-24-hour period of incubation with PPD-tuberculin. One such test kit is the BOVIGAM® (Thermofisher Scientific). An advantage of the test is its ability to detect early infections, allowing action to be taken before suspect animals become a source of infection to other animals. An advantage of the IFN-γ assay over skin testing, is that animals need only be captured once which is an important factor when dealing with wild or hard to handle animals (OIE, 2008).

M. bovis was diagnosed for the first time in goats in Australia in 1993. In that report the authors tested 19 goats. These were initially subjected to both the IDT and the IFN-γ test. Samples for culture were taken from one goat that had tested positive to both the IDT and IFN-γ test. The isolates produced by the culture were identified using PCR and the immunoperoxidase test (Cousins et al., 1993).

Gutiérrez et al., 1998 set out to evaluate cellular and diagnostic tests for the detection of *M. bovis* in goats. The experiment was performed 76 goats and the IFN-γ assay used in the diagnosis was found to have a sensitivity and specificity of 83.7% and 96% respectively (Gutiérrez et al., 1998). In a study conducted by Liébana et al 1998, the IFN-γ assay was found to be more sensitive than the IDT and was able to detect early onset infections in goats. This factor is important when considering TB eradication campaigns as goats can serve as reservoirs of infection and hamper TB eradication efforts (Liebana et al., 1998). Within the South African context, the DAFF does not recommend that a diagnosis be based on this test only (Department of Agriculture, 2016).

HI-BASED ASSAYS

Tuberculosis is often diagnosed clinically by skin testing or at necropsy. Unfortunately, these methods are unable to identify all infected animals. Serology offers an alternative to the identification of TB in animals missed by these tests (O'Brien et al., 2017). Tests based on the detection of antibodies such as the ELISA can augment the IDT rather than be an alternative to tests based on cellular immunity. The trait that makes ELISA an attractive test to use is its simplicity, but its major drawback is its limited sensitivity. This reduced sensitivity relates to the delayed and varying development of the humoral immune response during the disease. The specificity of ELISA can be increased by comparing antibody levels of bPPD and aPPD (OIE, 2008). Sensitivity and specificity based on the evaluation of 51 culture-positive samples and 25 culture-negative samples was 54.9% (28/51) and 88% (22/25) respectively when the tests were carried out in parallel with the IDT (Cousins and Florisson, 2005). Despite this low sensitivity, Gutiérrez et al 1998 suggested that the antibody-based ELISAs were useful as selective tests to be used on more advanced cases of TB in goats, as such cases tend to be more contagious and therefore have a greater impact on TB eradication success (Gutiérrez et al., 1998)

2.5 CASE REPORTS OF CAPRINE TB IN AFRICA AND EUROPE (1991 – 2018)

The focus for this section looked at studies or reports of confirmed TB cases in goats in Africa and Europe from 1991 to 2018. The literature searches were carried out on online databases such as Google scholar; CABI abstracts and PubMed but were not limited to these databases.

A spreadsheet was generated to capture the raw data and from this; charts, bar graphs and tables of the various factors were created and some of those are as follows:

2.5.1 AFRICA

TB in goats was mostly reported in Ethiopia followed by Nigeria. Together they represented 69.2% of reported TB cases in goats in Africa (1991-2018). Algeria, DR Congo, Eritrea and Sudan each had one reported case translating to 7.7% individually and 30.8% of reported incidences of goat TB. Sudan reported the largest number of goats diagnosed with TB (119 from 3200 that were tested) (G E and AO, 2017). The lowest number of TB cases reported in goats came from Ethiopia on two different occasions. One case was reported in 2007 (n=43) and another one in 2010 (n=170) (Amenu et al., 2010) (Tschopp et al., 2011). In Algeria in 2007 there were 60 (n=995) cases and in a separate incident in the DR Congo, 33 animals tested positive out of 656 animals that were tested. Finally in Eretria, there were 12 positive animals (n=876) (Ghebremariam et al., 2018) (Naima et al., 2011) (Naima et al., 2011).

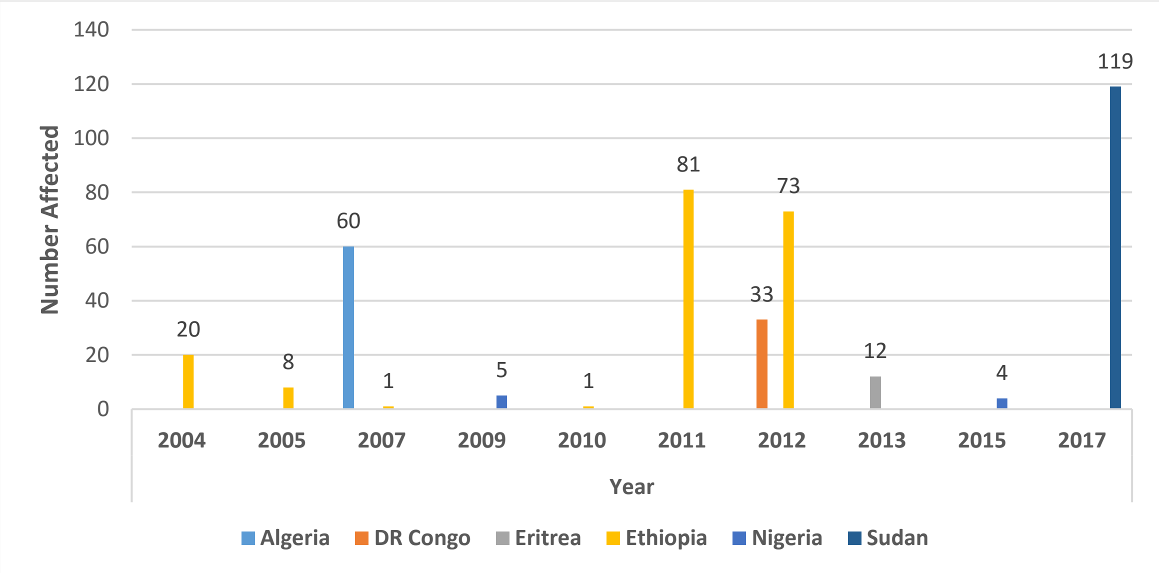

Figure 1: A bar graph of the number goats diagnosed with TB per country per year from 2004 to 2017

Cases of TB in goats in Africa were reported to be caused by *M. bovis* and *M. tuberculosis*. These were the only two species reported from all the articles encountered of TB in goats in the search period. *M. bovis* was most prominent, accounting for 61.5% of the reports.

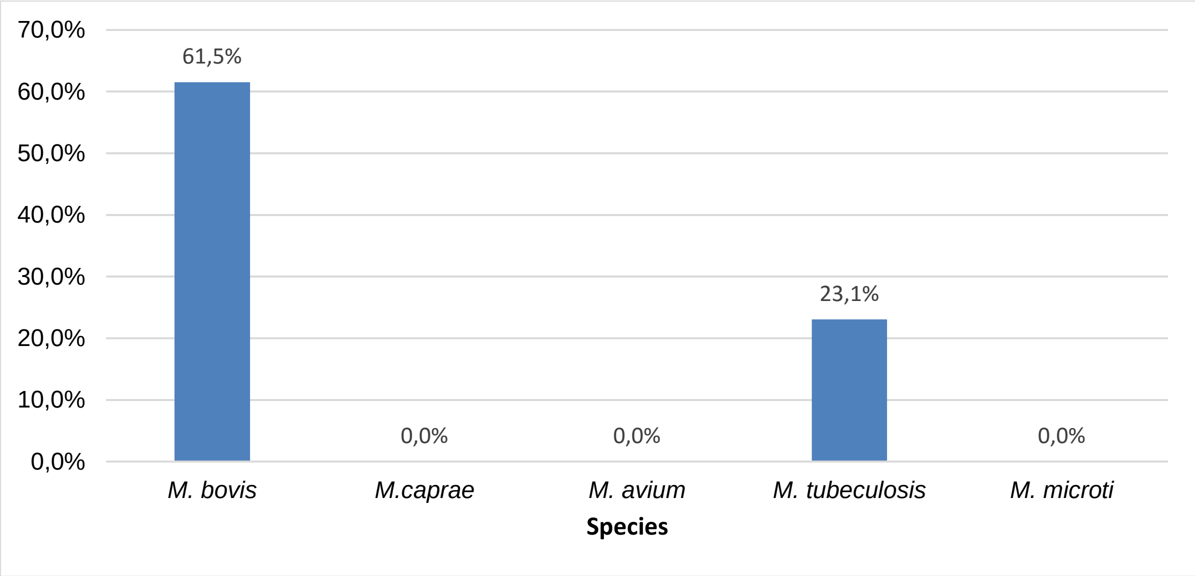

Figure 2: A bar graph of the causative organism of TB in goats in reported cases from Africa

As TB progresses and spreads throughout the body, it affects various organs, with that in mind; we sought to identify the organs affected during the disease process. Several organs were identified with the organ mostly affected being the lung (Fig. 3). It accounted for 76.9% of the reports. The liver and mesenteric lymph nodes accounting for 46.2% and 38.5% respectively. In all cases, the splenic and retropharyngeal lymph nodes were not affected.

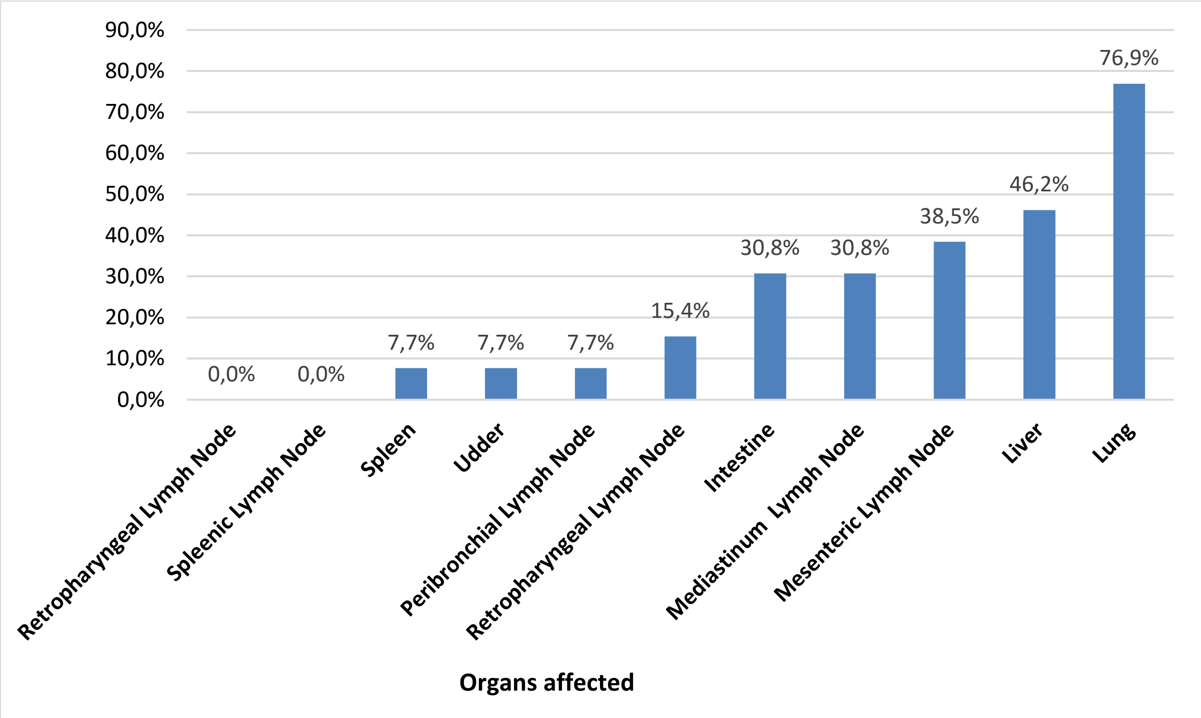

Figure 3: A bar graph depicting the proportion of organs affected by TB as reported in the TB cases in goats from Africa during the literature search

Various diagnostic techniques are available for the diagnosis of TB in goats. Four of the nine diagnostic tests were used in all the articles that were found in the literature. The most frequently used diagnostic test was diagnosis by post-mortem meat inspection. Proportionally it represented 77% of reported diagnostic tests; this was followed by post-mortem culture at 46%, histology at 38% and finally SIDT and CIDT both at 23% as shown in the graph below.

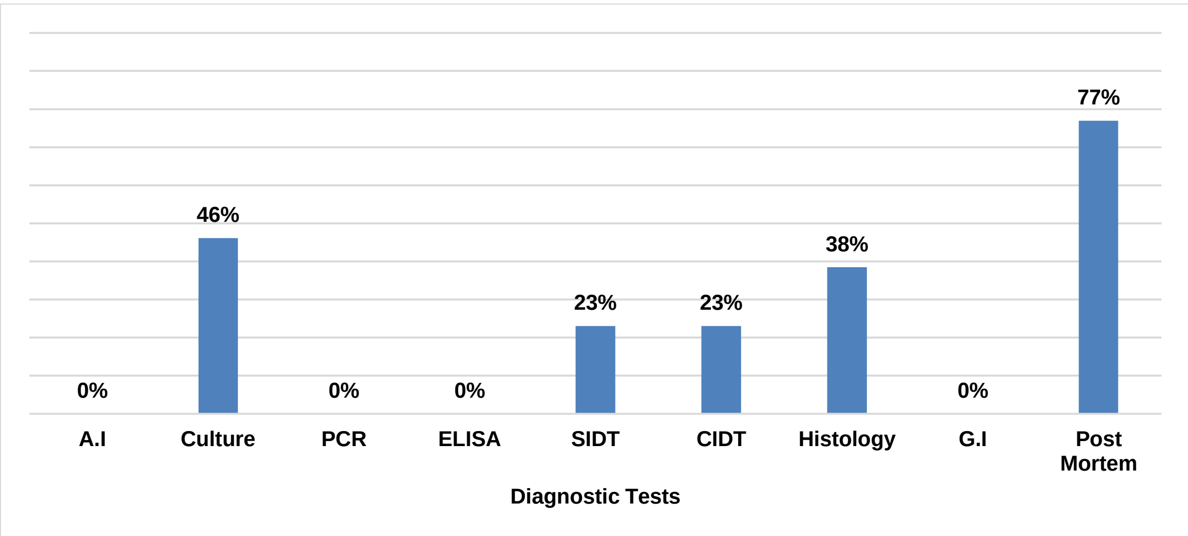

Figure 4: Diagnostic tests used in diagnosing TB in goats from 1991to 2018

2.5.1.1 SOUTH AFRICA

According to the search, there were no reports of TB in goats originating from South Africa in the timeline that the search was performed. The only report of TB in goats in South Africa was an incident that occurred at Onderstepoort, South Africa in 1928 (Fourie, 1928). In this report, a single goat was infected. The goat had been housed in a paddock that was separated from another paddock, which housed 12 clinically sick TB infected cattle. Following a post-mortem examination, it was found that the pharyngeal, bronchial and mediastinal lymph nodes were affected. Other organs that showed signs of disease were the lungs, bronchus and the spleen. Since then, no cases of bTB in goats has been reported in South Africa.

2.5.2 EUROPE

Most TB cases in goats were published from Spain (36%), followed by the United Kingdom (UK) (27%). Other countries that reported TB in goats include France, Ireland, Italy and Portugal (Bernabé et al., 1990) (Gutiérrez et al., 1995) (Crawshaw et al., 2008, Zanardi et al., 2013). The number of animals diagnosed in each incident are indicated in the graph (Fig. 5) below. The highest number of positive reactors occurred in Spain in 2008 in which there where 177 (n=177) positive reactors. In Spain in 1995, there were (n=42) positive reactors from (n=60) tested animals and again in Spain in 2013 101 goats (n=162) (Gutiérrez et al., 1995) (Álvarez et al., 2008) (Buendía et al., 2013).

In the United Kingdom in 2007 two animals were tested and both were positive for bTB, and in France in 2015 there were three positive reactors (n=3) (Quintas et al., 2010) (Álvarez et al., 2008) (Michelet et al., 2016) (Harwood, 2014).

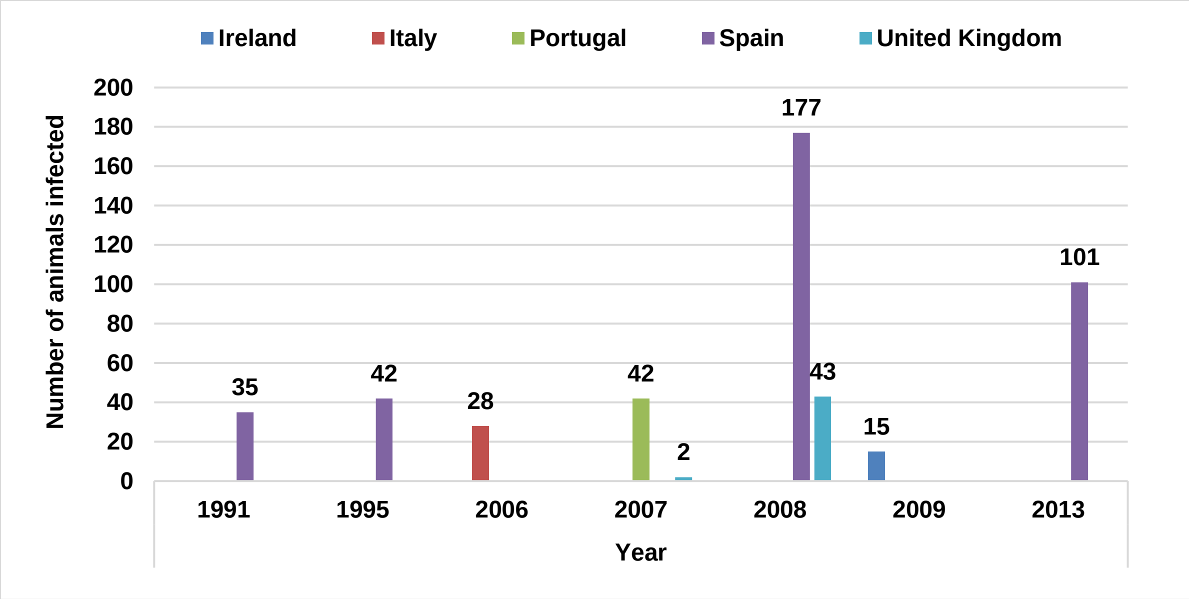

Figure 5: A bar graph representing the number of animals diagnosed in each of the TB incidents in goats in Spain, Portugal, Ireland, Italy and the UK

Overall, five TB causing organisms were identified from all the cases, *M. avium M. bovis, M. caprae, M. microti* and *M. tuberculosis*. The bar graph below (Fig. 6) represents the proportions of the organisms implicated in TB in goats.

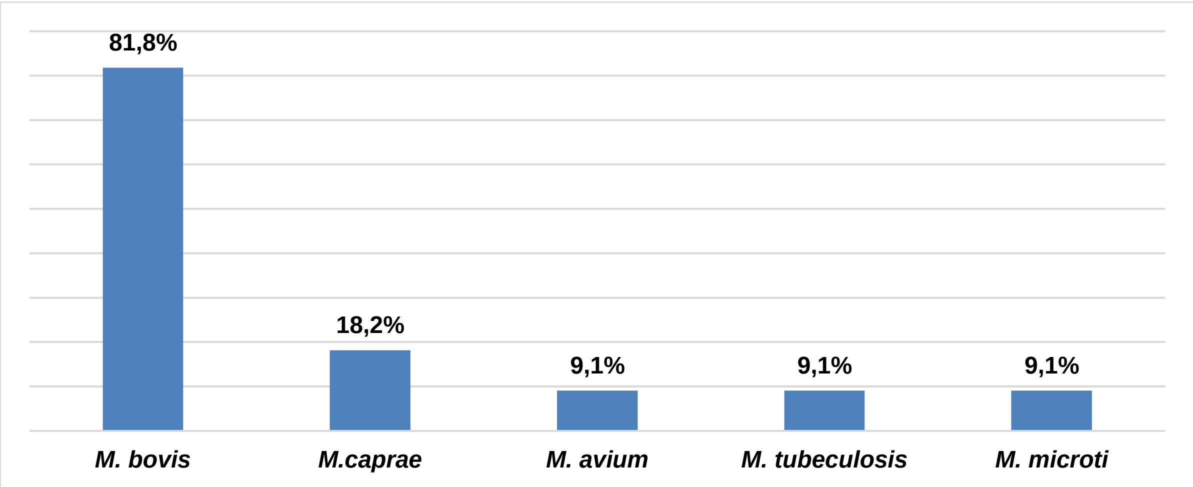

Figure 6: Proportional representation of organisms implicated in causing TB in goats (1991 - 2018).

Various organs were affected by the diseases process. The various organs that showed lesions characteristic of TB as stated by the authors in the various reports are represented Fig.7 below.

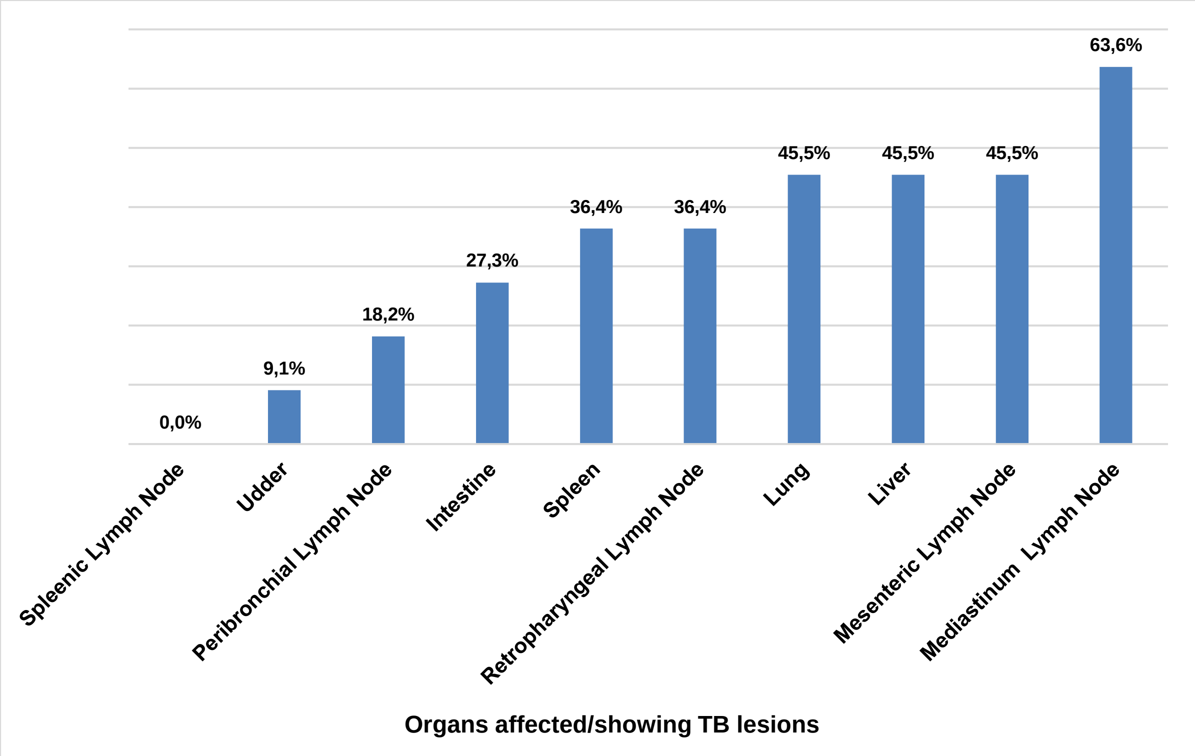

Figure 7: A bar graph depicting the proportion of organs affected by *M. bovis* as reported in the European cases found during the literature search. The organ that appears to be most affected is the mediastinal lymph nodes. Proportionally they accounted for 63.6% of affected organs. The lungs, liver and mesenteric lymph nodes were equally represented at 45.5% respectively. The least affected organ appears to be the udder (Álvarez et al., 2008) (Crawshaw et al., 2008) (Quintas et al., 2010) (Harwood, 2014) (Michelet et al., 2016).

The diagnostic techniques included animal inoculation (A.I), polymerase chain reaction (PCR), comparative intradermal testing (CIDT), enzyme-linked immunosorbent assay (ELISA), IFN-y, culture, histology, IDT and post-mortem (PM). The two techniques widely reported are the SIDT and PM. The least used diagnostic technique was PCR.

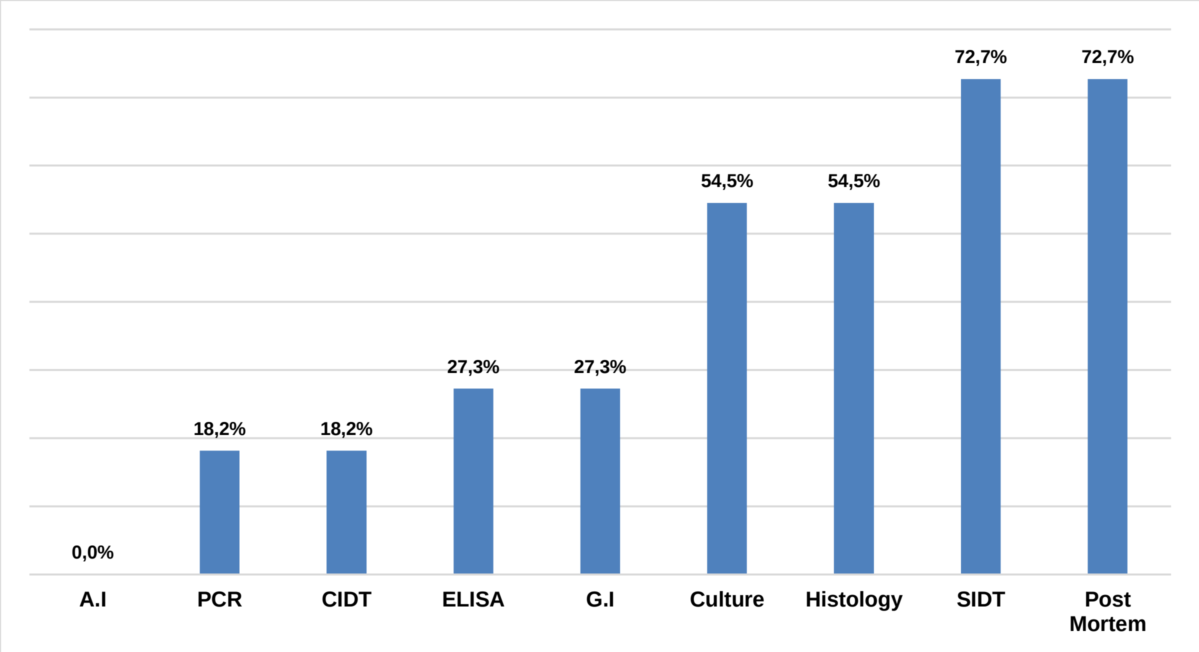

Figure 8: Proportional representation of diagnostic methods used in investigating TB in goats in Europe (1991 – 2018).

2.6 Discussion

In order to have a better understanding of TB in goats in South Africa and its potential impact on South Africa, we conducted a literature search of reported incidents of TB in goats in which goats were diagnosed as being infected with by mycobacteria from the MTBC. We searched for reports of confirmed TB cases in goats that reported from 1991 to 2018. The searched first focused on reports from Europe.

In Europe there were 11 reported incidents in which TB was positively identified in goats while in the same time period there were 13 reports of TB in goats in Africa. The motivation that led to TB being diagnosed in the two geographical regions are very different. While in Europe most of the reports were due to an ongoing investigation in TB in cattle which was found to have extended to include goats or was found to originate from goats (Crawshaw et al., 2008), (Sharpe et al., 2010), (Harwood, 2014) (Zanardi et al., 2013). In Africa, the majority of TB in goats reported stemmed from progressive searches centred around abattoirs (Adem and Agga, 2011) (Deresa et al., 2013), or animal research stations (Adem and Agga, 2011) (Tafess et al., 2011) (Kassa et al., 2012) (Tafess et al., 2011) (Tschopp et al., 2011) in which researchers were actively searching for TB in goats. This may mean that TB in goats in Africa may be under diagnosed as the reports from Africa are retrospective rather than prospective studies. The reports coming from Africa are due to organised, planned investigative efforts, which may mean that many cases go under diagnosed and unreported which is likely to impact TB control in cattle and have zoonotic consequences.

Reports from Europe identified five types of mycobacteria, that is *M. avium, M. bovis, M. caprae, M. microti and M. tuberculosis* but by far the most dominant was *M. bovis* accounting for 81.3% of identified mycobacteria. The species of MTBC diagnosed in goats from Africa were *M. bovis* (61.5%) and *M. tuberculosis* (23.1%). To date, *M. caprae* has not been diagnosed in goats in Africa. It has been largely limited to Europe. *M. caprae* is a subspecies of *M. bovis* that unlike *M. bovis* sub spp *bovis*, is pyrazinamide (PZA) sensitive which is used to differentiate the two (Niemann et al., 2002). The difference in the type of mycobacteria identified in the European and the African reports of TB in goats can be due to the availability of complex diagnostic tools such as spoligotyping, PCR and use of gene probes. This can be seen by the type of diagnostics used in the diagnosis of TB in the reports.

In all the reports from the African continent on TB in goats, none made use of PCR, as compared to investigations that occurred in Europe where PCR as a diagnostic tool accounted for 18.2% of the applied tools. Investigations from Europe utilised the full array of TB diagnostic tools available to science albeit in varying proportions. Investigations in Africa did not utilise tools such as PCR, IFN-γ assays and ELISA. It is therefore due to this difference in the availability and application of diagnostic tests that reports from Europe were able to identify more species of mycobacteria and those identified in reports from Africa. There are no validated diagnostic tools available for the diagnosis of TB in goats. The OIE has only published diagnostic guidelines for cattle. Due to the lack of standardisation, it becomes difficult to accurately compare the outcomes of any two situations.

The distribution of lesions between the occurrences was somewhat different. Although the organs affected that appeared from European reports also occurred in the Africa reports, there were some differences. The lungs were the most widely reported organ affected in reports from Africa while mediastinum lymph nodes were the most reported organ affected in European cases.

There were no published incidents of positively diagnosed TB infection in goats from South Africa. There are several possibilities that can explain this outcome. Firstly, and in the authors view less likely, is that there simply are no infected goats in the country. This is least possible because TB in goats has been diagnosed in goats in other countries that have similar husbandry methods and comparable cattle prevalence's. An example of such a country is Ethiopia. The conditions conducive for and allow for the transmission of *M. bovis* between infected cattle and goats and between goats exist in South Africa. At night, goats are kept in usually small, poorly ventilated housing alongside cattle for protection from predation.

However, low prevalence's of TB in goats have been recorded in situations in which goats and cattle are herded together (Tschopp et al., 2011). In the study carried out by Tschopp et al 2011, they found a very low prevalence (0.4%) of TB in goats despite the intensive relationship that existed between cattle and goats. In general, it is suggested that due to the difference in the feeding habits between cattle and goats, transmission through infected pasture and close contact during feeding may not afford enough opportunity for transmission of pathogen. Cattle are grazers in nature while goats are browsers. A plausible explanation to the low prevalence of TB in goats that Tschopp et al 2011, found in their study was the fact that the turnover of small ruminants such as goats is high. Unlike cattle that are usually kept for a longer time, goats are usually kept for a short of period as they are either slaughtered or traded off. This can limit the time of exposure and therefore the development of disease in goats, which can appear as a resistance to disease while in fact it is due to a lack of sufficient time to allow for the infection to result in the development of the disease (Tschopp et al., 2011).

The second possible reason as there were no published cases of TB in goats in South Africa, is that, perhaps TB was being diagnosed on the ground but simply not published. For a long time goats were considered to be resistant to infection by MTBC (Tafess et al., 2011). This can create an inherent bias in a veterinarian's mind to be less likely to consider TB in goats. However, considering the paucity of TB in goats, it would be reasonable to expect that diagnosed cases in the field would be worthy of a published in a peer-reviewed journal or reported online. While TB in goats was mentioned in the Bovine TB scheme manual of 2016 (Department of Agriculture, 2016) there were no guidelines on the actual testing of TB in goats.

In November 2018, the DAFF released a standalone TB testing manual specifically directed toward TB testing in goats, sheep and new world camelids. In the manual, the procedures for TB testing are explained in detail. Therefore prior to November 2018, TB testing in goats was not a priority and perhaps could help to explain the absence of reports of TB in goats (Department of Agriculture, 2016).

Chapter 3

MATERIALS AND METHODS

3.1 POPULATION AND SAMPLING

The population of the study were state veterinarians who were involved in monitoring-controlled diseases and employed by DAFF. It is a department in the government of South Africa. This department oversees monitoring and regulating the agricultural sector in the country, making sure the nation has access to safe and wholesome food products.

Convenience sampling was used to determine the number of participants. Potential participants were contacted either via email or telephonically or in person and those that expressed an interest and where available to participate where selected for the study.

3.2 MATERIALS AND METHOD

A survey study design using a quantitative research approach was used. Data were collected through face-to-face interviews using a semi-structured survey questionnaire.

Contacts of state veterinarians where obtained from the DAFF website. Participants were initially contacted via email. In the email the participants were given a brief introduction of the researcher as well as his intention of performing the questionnaire. The consent form as well as the questionnaire were attached in the email. If the participants did not respond in a week, a follow up email was sent as a reminder. If the participants did not respond to the reminder email, efforts were made to contact them telephonically.

Willing participants would either do so via email in which they would read and sign the consent form and then proceed to answer the questionnaire. They could either fill in with a pen or, type in their responses electronically.

During the face-to-face interviews, participants were given the option to fill in the questionnaire themselves or let the interviewer read out the questions and fill in their responses for them. They were however requested to go through the consent form and fill it in before the interview could start. Other participants preferred to go through the questionnaire themselves while in the presence of the interviewer.

Chapter 4

Results, Discussion and Conclusion

4.1 Results

In the first question respondents were asked to identify the province in which they operated. Eight out of the nine provinces that constitute the Republic of South Africa were represented in the responses. There were no participants from the Western Cape province as none responded to requests for participation either via telephonic interviews or face-to-face interviews. The percentage distribution of respondents is illustrated in Fig 9 below.

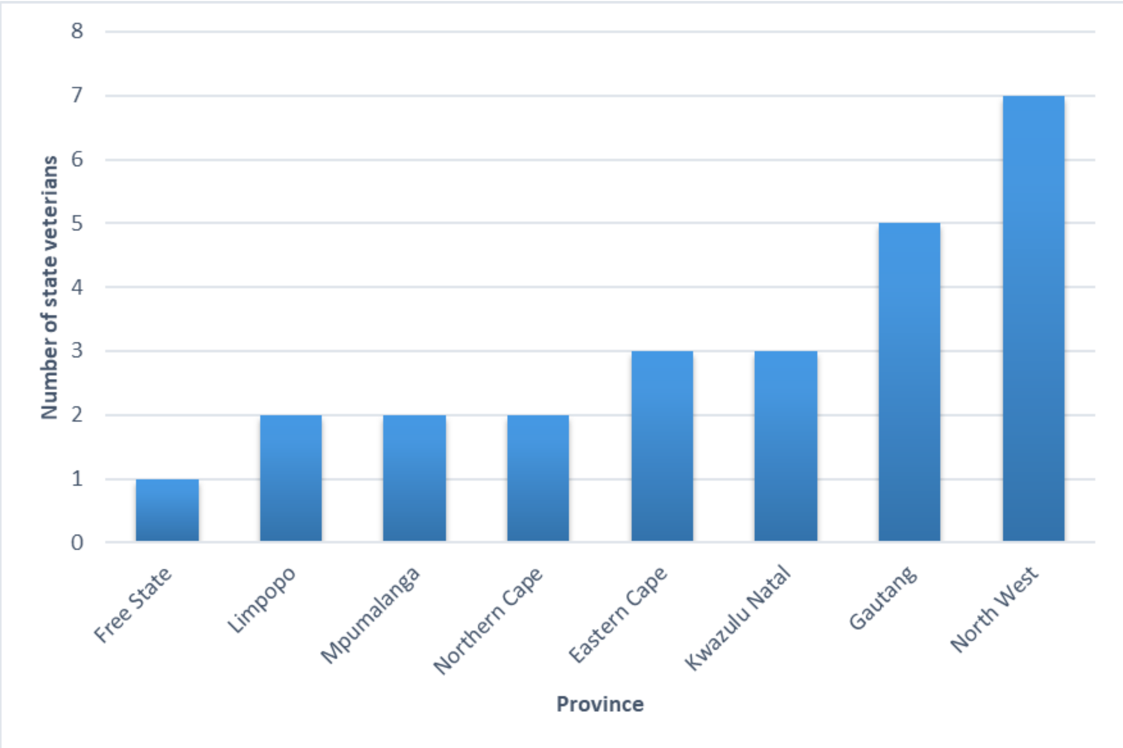

Figure 9: Distribution of state veterinarians by province. The North West had the highest representation with 7 participants. It was followed by Gauteng that had 5 participants, followed by the Eastern Cape and KwaZulu-Natal both with 3. The Northern Cape, Mpumalanga and Limpopo each had 2 participants each. The Free State province had the least representation of 1 participant

In the second question, participants were asked if TB testing had been conducted in cattle or goats in the past 6 months. Out of the 25 participants, seven did not know if testing had been done in either species. Eighteen participants confirmed that testing had been done in cattle. None of the participants had ever tested for TB in goats in any time period. The North West recorded the highest amount of activity with respect to TB testing in cattle with a percentage of 25%, followed by Gauteng at 20%. Eastern Cape and KwaZulu-Natal each had 15%; Northern Cape had 10%; Limpopo, Mpumalanga and Free State recorded 5% each.

All participants were asked how often TB testing was performed in cattle (Fig. 10), and approximately 25.9% did not know how frequently this was done in their respective provinces.

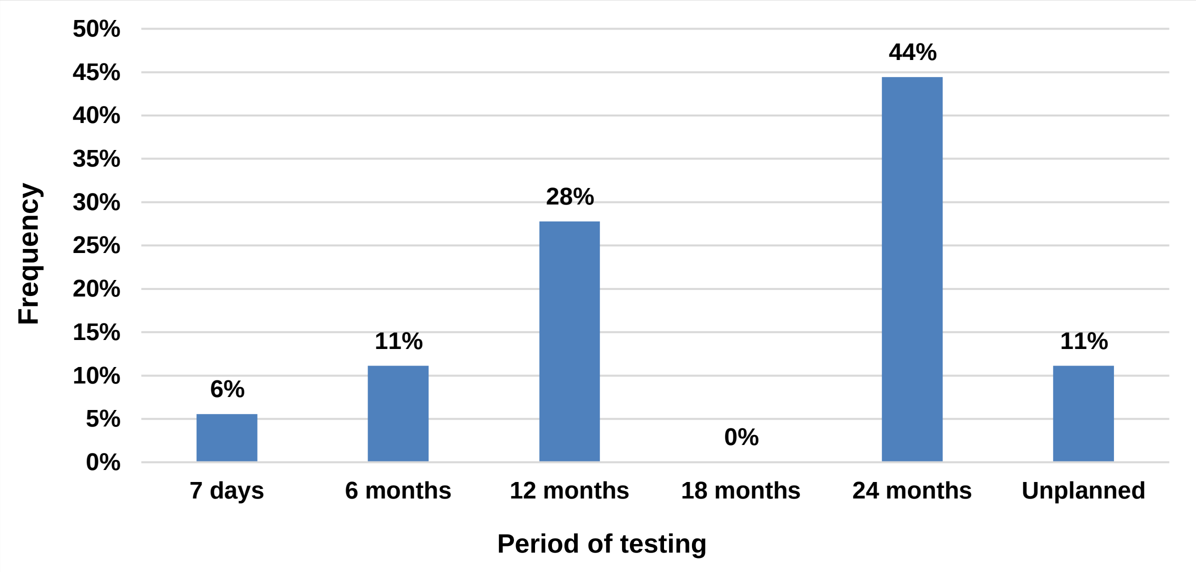

Figure 10: The graph above shows the frequency of TB testing in cattle. The most widely reported frequency of TB testing is every 24 months followed by every 12 months then Unplanned and every 6 months at the same level. The lowest being every 7 days

On the question of TB prevalence in cattle, 56% of the participants did not know the prevalence of TB in cattle in their provinces of operation and 32% described the prevalence as low without assigning a figure to it. Eight percent indicated the prevalence in their region was 0, and 4% of the respondents said the prevalence was 8%.

Province	0	8	low	N/K
Eastern Cape	0	1	0	2
Free State	0	0	0	1
Gauteng	0	0	0	5
KwaZulu Natal	0	0	1	2
Limpopo	0	0	0	2
Mpumalanga	0	0	1	1
North West	2	0	4	1
Northern Cape	0	0	2	0
Grand Total	2	1	8	14

Figure 11: Tabulated responses to the question on the prevalence of TB in cattle in the respective provinces of the respondents. Two participants reported a prevalence of 0, 1 reported a prevalence of 8%, 8 simply referred to the prevalence as 'low' while 14 stated that they did not know the prevalence represented by 'N/K'.

All the respondents (n=25) indicated that TB testing of goats was not done, and neither were there cases or reports of TB detected in goats. However, participants (54%) indicated that goats were kept in close contact with cattle or wildlife in their respective areas. Majority of the participants (92%) indicated that both cattle and goat carcasses were examined for TB lesions at the abattoirs in their regions.

From the questionnaire it was clear that the respondents believed that TB in goats was being overlooked (76%) from a disease control point of view.

Reasons for not performing TB testing in goats included low prevalence of bTB in goats (15%), limited resources (25%), not mandatory (55%) and the remaining respondents indicated other reasons such as the lack of specific testing protocol for goats (Fig 12).

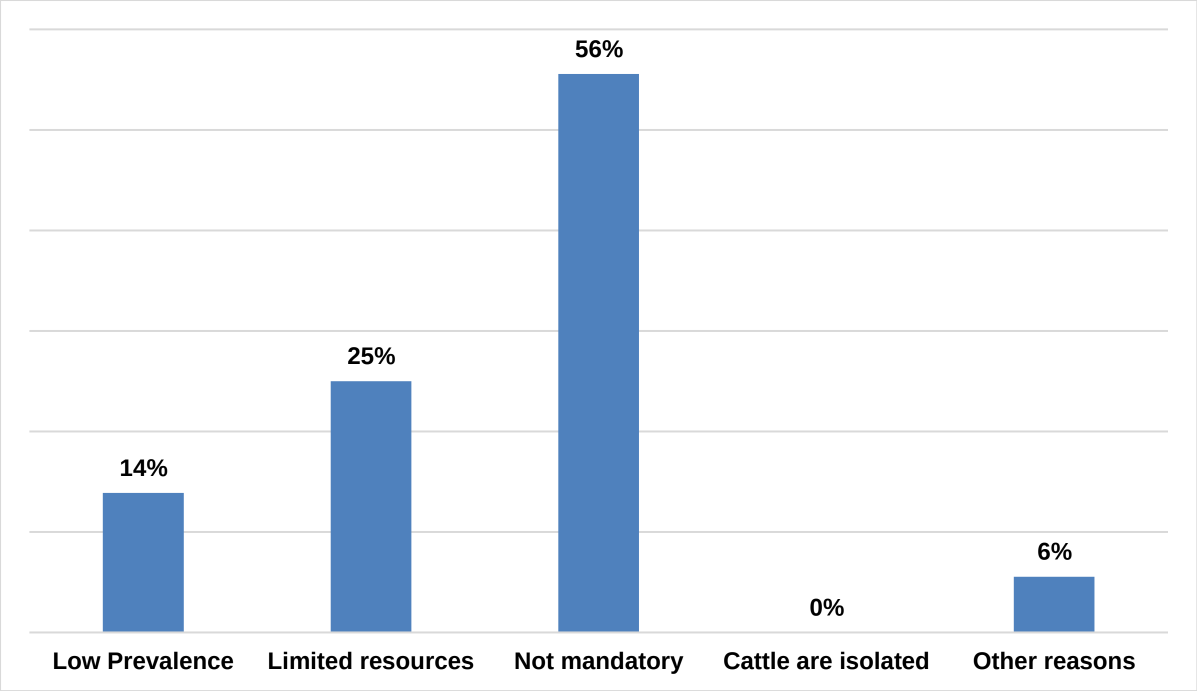

Figure 12: The graph above indicates the reasons provided by the participants for not testing for TB in goats.

The CIDT was by far the most recommended test to use to diagnose TB in goats making up for more than 50% of the responses. The second most recommended diagnostic tool was IDT accounting for just over 40% of recommendations. Finally, the least popular diagnostic tool was the IFN-γ assay.

Participants were asked if goats or goat related products were tested for TB before entering the market. In the follow up question participants were asked to name the test and its validation status. 82% of the participants said no, and 18% said yes to goat or goat related products being tested for TB. The tests said to be used were visual inspection, culture and the IDT. However, none of the respondents were able to say if the tests were validated or not for use in goats. All the participants agreed that knowing the prevalence of TB in goats would help efforts to control TB in cattle.

4.2 DISCUSSION

This study was performed to determine the status of TB testing in goats in South Africa. To achieve this aim, using a semi-structured questionnaire, the study gathered data from state veterinarians about TB in goats, the tests that are used for diagnosis and the validation status of these tests that may be used in goats for diagnosis of TB.

Eight of the nine provinces of South Africa were represented in the results. None of the participants tested goats for TB and several participants (n=25) did not know if testing was being conducted at all. There were no participants from the Western Cape, thus no information on TB testing in goats has been gathered from this province. Thus, part of the objective was not fully satisfied as the data presented in this study does not represent data from all the provinces. A possible explanation for no representation from the Western Cape could be that veterinarians did not have the time to respond or were not permitted to respond and thus thought it safe not to participate.

The results from the questionnaire indicates that not much is being done in terms of testing goats for TB and there is a general lack of awareness on the state of TB testing in cattle among state veterinarians from the provinces included in this study. In cases where cattle and goats are grazing together or sharing resources, it was expected that there was some level of testing being done in both species due to the potential spread of TB between goats and cattle that share pasture.

The questionnaire asked for testing in each time period and it is possible that testing may have been performed outside the given time period and so this information was missed by the questionnaire.

It is also worth noting that none of the participants from the eight provinces captured in the questionnaire performed TB testing in goats in the last 6 months. The results obtained from the questionnaire gives the impression that TB testing in goats is overlooked though TB is known to exist in goat populations, as presented in the previous chapter. Another possibility is that there were no reports of TB testing in goats due to the absence of the disease in goats and hence no need of testing for it. Extensive work in being carried out in Ethiopia where researchers are trying to establish the prevalence of TB in goats in different regions of the country. These studies are mainly conducted at post-mortem and the prevalence of TB in goats are as high as 3.5% (Tafess et al., 2011).

The questionnaire also sought to obtain information on TB testing intervals in cattle. We found that the most reported TB testing interval among participants was every two years while the least reported was weekly testing (strategy unknown). Although no mention is made in the TB testing manual on the frequency, every two years seems to be the most widely reported testing interval.

Frequency of TB testing is a function of risk, geography and intended use. The responses from participants bridged provinces such that the results were not based according to province. One could argue that this variation could be due to a lack of a TB testing interval policy and so each region and office are forced to come up with their own testing intervals influenced by factors such as risk of spread of TB from cattle to goats, and resource availability. This could be reflective of the different risk levels within and between provinces which in turn drives the necessity and frequency of testing. Testing of TB in the United Kingdom follows similar patterns in which frequency of testing is determined by the regional level of risk. According to the Animal and Plant Health Agency (APHA), the default TB testing intervals for cattle are in low-risk Areas is 48 months and for herds in the high-risk areas and parts of the edge area is 12 months. The edge area is the buffer zone between the high-risk zones and the low risk zones. Certain countries (or parts of counties) in the edge area are on a six-monthly testing frequency, www.gov.uk/guidance/bovine-tb-testing-intervals-2019.

Long TB testing intervals in cattle can allow for prolonged cross infection between cattle and goats. Frequent testing allows for early detection and implementation of corrective measures thereby reducing the chances of cross infection between cattle and goats. Some participants indicated that they had not performed any TB testing in the last 1 - 3 years due to a lack of resources.

During the TB outreach programme, hosted by the Faculty of Veterinary Science at the University of Pretoria on the 2nd of March 2015, attended by veterinarians, wildlife managers, regulatory agency staff and other interested parties, Dr Sibanda (DAFF) was quoted that government was considering a number of proposals in order to improve the control of TB in the country. Dr Sibanda went on to write, "There will be two initial tests at three-month intervals performed by the state and testing every two years at the owner's expense. The surveillance program (old diagnostic herd test) will provide information on prevalence, and costs of mini campaigns will be covered by the state. Testing in cattle will be performed at five year intervals (except if animals are at risk)", www.up.ac.za/media/shared/678/btb-outreach-day-booklet-print_03042017.zp114198.pdf. The results of the questionnaire appear to contradict the suggested testing intervals as neither the three monthly nor the five yearly intervals were reported by our participants.

To ascertain the level of disease in cattle from the veterinarian's point of view, we asked if they knew the prevalence of TB in cattle in the provinces in which they operated. The results pointed to a lack of knowledge in the aspect as reflected in the results. According to the results we obtained, 56% indicated that they did not know the prevalence of TB in cattle in their provinces while 32% described the prevalence as 'low'. On the other end of the scale, 8% described the prevalence in their provinces as 0 while 4% described it as 8%. The responses were not divided along province lines as participants from the same province would answer differently to the same question. The lack of consistency and apparent lack of knowledge on the issue support claims made by Nyamakwere et al where they say DAFF, sub-Directorate Veterinary Public Health lacks a microbiological monitoring programme (Nyamakwere et al., 2016). The results from the questionnaire are further supported by the lack of official figures with respect to TB in cattle. It would appear that TB in cattle have largely been overlooked until it was detected in buffalo which eventually led to the introduction of the national bovine TB control and eradication scheme in 1969, which facilitated a decline in bTB (Michel et al., 2009). Despite this development, the TB control program has been dogged by problems among them manpower shortages, budget constraints and disease prioritization (www.up.ac.za/media/shared/678/btb-outreach-day-booklet-print_03042017.zp114198.pdf). Instead of providing a figure with respect to the prevalence in their provinces, some participants indicated that due to a lack of resources they had not been able to test for TB and so had no information to provide.

When asked on TB testing or diagnosis of TB in goats, none of the participants (n=25) had come across TB in goats nor knew of testing in goats. The results clearly show that TB testing is not being carried out in goats in the field.

At the time of the survey, cattle were the focus of the TB control program though it was acknowledged that goats may play a role in the epidemiology of TB and should be tested. In the Bovine TB scheme Manual goats are described as being susceptible to *M. bovis, M. avium and M. tuberculosis* and can develop disease if sufficiently challenged. The manual advises veterinarians to check TB in goats in situations in which the disease is found in cattle and if there is close habitation between the two species (Department of Agriculture, 2016). Conditions conducive for the transmission of TB between cattle and goats do exist as shown by the literature study performed in this project. We asked practitioners if they had come across situations where there was close habitation between cattle and goats and 54% of the participants responded yes to this question. According to the Newsletter: National Livestock Statistics published by DAFF in August 2018, it was estimated that there were 5 475 000 goats in South Africa. Considering that 5 475 000 is a large number, we were expecting to have some reports on confirmed incidents of TB in goats, but we did not, as shown by our results. A possible explanation to this could be that the focus of TB control in South Africa is heavily placed on cattle, and for good reason since cattle are regarded as the domestic reservoir species of the pathogen. Another possible but less likely explanation is that there were truly no infections of TB in goats. It is less likely because TB in goats has been diagnosed in other regions of Africa such as Ethiopia with similar TB trends in cattle and similar husbandry methods as those in South Africa, that expose goats to TB diseased cattle.

In an interesting development, DAFF released a manual entitled “Tuberculosis testing in sheep and goats”, whose purpose is to provide guidelines on the testing of small stock. The release of the document comes after our questionnaire had been circulated among veterinarians. We would like to believe that its release was a direct response to questions posed by the survey from this project.

Most participants expressed the view that TB in goats was being overlooked from a disease control point of view. This is evident from our results as they show that 76% agreed to TB being overlooked when it came to disease control. Asked on the reasons for not performing TB testing in goats, 55% responded by saying TB testing in goats was not mandatory while 25% cited limited resources as the reason. A brief history was provided during the bTB outreach day jointly held by the universities of Stellenbosch and Pretoria. One of the representatives wrote that, challenges have always been part of the control program. These problems ranged from severe staff and vehicle shortages, disease prioritization, funding problems to the availability of tuberculin, www.up.ac.za/media/shared/678/btb-outreach-day-booklet-print_03042017.zp114198.pdf.

This study was a first of its kind and provided insight into the status of TB testing in goats in South Africa and indicated a few challenges by the DAFF in doing more to test other livestock for TB.

4.3 Conclusion

The aim of this project was to determine the status of TB testing in goats in South Africa after having realised the potential of spread of TB from cattle to goats through husbandry methods that allow for the close habitation of the two species. To achieve this aim, a questionnaire was designed and used as the instrument to collect data on this topic.

In addition to this, an extensive literature search was conducted on reports of confirmed cases of natural infection of TB in goats. The search looked at reports from Europe and Africa from 1991-2018. From the results of the project, taking into consideration information gathered from the questionnaire survey as well as the case studies of reports of TB in goats, we can conclude that TB testing in goats is not performed in South Africa. We can also conclude that there is a lack of knowledge among veterinarians when it comes to issues surrounding TB testing in both cattle and goats. TB in goats' remains largely ignored judging from the lack of knowledge on the subject and reports from literature.

The release of a separate addition to the Bovine Tuberculosis Scheme Manual of December 2013 entitled "Tuberculosis testing in sheep and goats manual" was released in November 2018. This manual was released after the researcher had completed the questionnaire interviews. It is very likely that this TB testing manual was released in response to the questions raised in the questionnaire and realisation of the shortcomings in the original Bovine TB Scheme Manual where primary focus is on TB testing in cattle.

A major limitation in the study was of the lack of participation from veterinarians. The level was low regardless of the numerous emails that were sent out, and few chose to participate in the study. In hindsight, it would have been more rewarding to make the questionnaire more user friendly than its current version. Some participants expressed interest in the survey but said the questionnaire was not in a user-friendly format. Perhaps conducting the questionnaire via programs such as SurveyMonkey (surveymonkey.com) would have made participation easier. Another limitation to the study was the lack of input from animal owners themselves. Accommodating their points of view would have provided a clearer picture on the subject matter.

Looking into the future of TB testing in goats, I highly recommend sero-surveillance studies to be carried out in order to determine the extent of TB in goats. Such a study would focus in areas where the prevalence of TB in cattle is high and where the husbandry practices support the likelihood of transmission of TB from cattle to goats. Epidemiological studies can be carried out in regions where TB in cattle continues to be a problem despite control measures. The focus in those areas would be on the possible role of goats as reservoirs of infection for cattle.

ANNEXURE 1

CONSENT FORM FOR PARTICIPATION IN RESEARCH PROJECT TITLED:

Questionnaire-based study on the state of tuberculosis testing in goats in South Africa

Name of Researcher: Dr Nyoni G (**Supervisor**: Dr Morar-Leather D)

I agree to participate in the above-mentioned research project led by Dr Godfrey Nyoni from the University of Pretoria, Faculty of Veterinary Science.

The purpose of this document is to specify the terms of my participation in the project through being interviewed.

1. I have been given sufficient information about this research project. The purpose of my participation as an interviewee in this project has been explained to me and is clear.

2. My participation as an interviewee in this project is voluntary. There is no explicit or implicit coercion whatsoever to participate.

3. Participation involves being interviewed by researcher(s) from the University of Pretoria. The interview will last approximately 15 minutes.

4. I have the right not to answer any of the questions. If I feel uncomfortable in any way during the interview session, I have the right to withdraw from the interview.

5. I have been given the explicit guarantees that, the researcher will not identify me by name or function in any reports using information obtained from this interview, and that my confidentiality as a participant in this study will remain secure.

6. I have been given the guarantee that this research project has been reviewed and approved by the ethics Committee of the Department of Health Science at the University of Pretoria.

7. I have read and understood the points and statements of this form. I have had all my questions answered to my satisfaction, and I voluntarily agree to participate in this study.

Participant Signature Date

Researchers Signature Date

ANNEXURE 2

PROJECT TITLE

Tuberculosis in goats in South Africa; prevalence, zoonosis, diagnostics and industry

AIM

To establish if there is a need to perform TB testing in goats in South Africa, and if there is a need, to determine why there is a lack of TB testing in goats,

OBJECTIVES

In order to achieve the aim of the study, the research objectives are as follow:

To ascertain the knowledge of state veterinarians about caprine TB testing

To identify tests used for caprine TB

To determine the validation status of TB diagnostic tests used in goats in South Africa.

QUESTIONNAIRE: BOVINE TUBERCULOSIS TESTING OF CATTLE AND GOATS

This questionnaire is designed to find out about TB testing of goats and cattle in your area. Please answer the questions truthfully.

1. In which province are you a State Vet

- Gauteng
- Limpopo
- KwaZulu-Natal
- Free State
- Western Cape
- Northern Cape
- North West
- Mpumalanga
- Eastern Cape

2. In the last six months was TB testing performed in
(Tick if appropriate)

- Cattle
- Goats

3. How often is TB testing done in cattle?

----

4. What is the prevalence of bTB in cattle in your area of operation?

----

5. How often is TB testing done in goats?

----

6. Was bovine TB ever detected in goats in your area?

(Tick the appropriate box)

- ○ Yes
- ○ No

7. If yes, how was it diagnosed?

----

8. What is the prevalence of bovine TB in goats in your area?

----

9. Is the area where the goats are kept in close contact with wildlife and or other livestock that have TB?

(Tick the appropriate box)

- ○ Yes
- ○ No

10. At the abattoir in your region are the cattle and goat carcasses examined for bovine TB lesions?

(Tick the appropriate box)

i. Yes

ii. No

11. Do you believe TB in goats in being overlooked from a disease control point view?

----

12. What are the reasons for not performing TB testing in goats?

(Tick the appropriate box)

- ○ TB prevalence in the area is low

- Limited recourses
- Not mandatory
- Cattle do not mix with other livestock
- Other reasons not mentioned here

13. Which tests do you recommend for testing TB in goats?

----

14. Why do you recommend the above mentioned test/s

----

15. Are imported goats or related products tested for TB before entering the market? (Tick the appropriate box)

- Yes
- No

16. If yes, which tests are used, please indicate if the test have been validated for use in goats

....Yes/No

....Yes/No

....Yes/No

17. Having knowledge that goats can be infected with bTB and spread the infection to cattle, would determining the prevalence of bTB in goats help efforts to control bTB in cattle?

(Tick the appropriate box)

- Yes
- No

ANNEXURE 3

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

Ref: V121-17

30 November 2017

Dr. D Morar-Leather
Department of Veterinary Tropical Diseases
Faculty of Veterinary Science
(darshana.morar@up.ac.za)

Dear Dr Morar

V121-17
Tuberculosis in goats in South Africa; prevalence, zoonosis, diagnostics and industry (G Nyoni)

The committee has evaluated your project. Since the project is not using animals or animal samples, you do not require animal ethics approval.

If you have any questions, please feel free to contact the committee.

Yours sincerely

Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee
Copy: DN Qekwana (Researcher)

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Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

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