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A comparison of perceptions of the tuberculin skin test and an incentive postmortem-based surveillance system in the Mnisi community, Mpumalanga, South Africa

By

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DECLARATION

I, Marange Rudo, 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.

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Signed……………………………………

Marange Rudo

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LIST OF ABBREVIATIONS

AEC	Animal Ethics Committee
AIDS	Acquired Immuno Deficiency Syndrome
bTB	Bovine tuberculosis
CMI	Cell mediated immunity
DAFF	Department of Agriculture Forestry and Fisheries
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organisation
KNP	Kruger National Park
HIV	Human Immunodeficiency Virus
IFN-y	Interferon-gamma assay
MTBC	*Mycobacterium Tuberculosis* Complex
OIE	Oficina Internacional de Epizootias
PCR	Polymerase Chain Reaction
PMS	Postmortem surveillance
PPD	Purified Protein Derivatives
SICT	Single intradermal comparative tuberculin test
SIT	Single intradermal tuberculin test
STATSSA	Statistics South Africa
TB	Tuberculosis
TST	Tuberculin skin test

ABSTRACT

Tuberculosis (TB) is a global health concern. *Mycobacterium bovis (M. bovis)* and *Mycobacterium tuberculosis (M. tuberculosis)* are the most common causes of TB in animals and humans respectively. As part of TB control strategies most governments have instituted test and slaughter policies to eradicate bovine TB (bTB). While this has been met with some success, innovative and effective strategies to control TB are needed. We evaluated the postmortem surveillance (PMS) system as an alternative to the tuberculin skin test (TST) and found it to be a potentially cheaper and effective surveillance method. The level of TB awareness in the Mnisi community was also evaluated. Tuberculosis awareness by the community is also an effective way of TB control as education empowers people to make informed choices with regards to mitigating TB risk factors in their daily lives.

CHAPTER 1

Literature review

Introduction: Importance of Bovine Tuberculosis

Mycobacterium bovis (M. bovis) is the causal agent for bovine tuberculosis (bTB), an infectious and chronic disease of livestock, wildlife and humans (O'Reilly & Daborn 1995a; Neill, Skuce & Pollock 2005). *M. bovis* is a significant pathogen due to its conservation threat (wildlife infections), economic implication (cattle disease), and zoonotic importance.

Zoonotic tuberculosis is a global public health concern, especially in developing countries due to the paucity of funds to control infectious diseases and deficiencies in preventive and/or control measures (Etter *et al.* 2006; Olea-Popelka *et al.* 2017). In developed countries, such as Australia, bTB eradication has been successful because of the implementation of sustained bTB eradication and control measures such as testing, culling and pasteurisation of milk (More, Radunz & Glanville 2015). In low-income countries such as in Africa, bTB is prevalent in cattle populations (Ayele *et al.* 2004). Losses in animal production through carcass condemnations at abattoirs, decreased milk yields and low meat yield resulting from emaciation and poor reproductive performance due to bTB negatively impacts the African economy. The impact of loss in animal production or death of a single animal has far reaching economic and cultural consequences to subsistence farmers in poor communities (Olea-Popelka *et al.* 2017). One's social standing in the community is partly determined by the number of livestock an individual owns (Randolph *et al.* 2007).

Bovine TB has also been implicated in the increased cases of human health problems in African countries (Cosivi *et al.* 1998; Müller *et al*. 2013). In association with the pandemic of human immunodeficiency virus (HIV), an increase in human TB due to *Mycobacterium tuberculosis (M. tuberculosis)* has been noted, and this may suggest a similar increase in human TB cases due to

M. bovis (Grange & Yates 1994; Müller *et al.* 2013). In Africa this is crucial as human-animal interactions are intensive, people live in close proximity with cattle and or other livestock and the standard of hygiene during handling of animal products is usually very low (Ameni & Erkihun 2007). Thus, there is a need to sensitise livestock owners on endemic livestock diseases and the risks they pose, so as to gain their support as counterparts for extension services and disease control programs. Disease monitoring and eradication are activities that should use multi-partner (stakeholders inclusive) and multi-disciplinary approaches. Policy makers hence need to take into cognisance the level of bTB awareness by cattle farmers when considering mitigation and effective implementation of control strategies (Munyeme *et al.* 2010).

Bovine TB mitigation in cattle populations can eradicate or drastically reduce the disease in human communities (Sudre, ten Dam & Kochi 1992; Cosivi *et al.* 1998; Hlokwe *et al.* 2014). In South Africa due to control strategies implemented since 1969, bTB prevalence has been reduced to less than 1% (Hlokwe *et al.* 2017). As bTB remains a worldwide problem, it is therefore, imperative to intensify control and preventive measures in livestock aimed at its eradication (Humblet, Boschiroli & Saegerman 2009a). Since a distinction between the *Mycobacterium* species, i.e. *M. bovis* and *M. tuberculosis,* is not systematically and routinely performed, the incidence of *M. bovis* infection in humans remains underestimated. As the real incidence of *M. bovis* on human health is still unknown it is essential to push for the advancement of the eradication of bTB worldwide using innovative programmes, especially in developing countries with limited resources (Humblet, Boschiroli & Saegerman 2009a; Olea-Popelka *et al.* 2017).

1.1 Tuberculosis aetiology

The *Mycobacterium tuberculosis* complex (MTBC) consists of mycobacteria that cause TB in their hosts (Wirth *et al.* 2008; Egbe *et al.* 2017). Mycobacteria are non-spore forming, non-motile aerobic rods. Their cell walls have a high lipid content and are thus difficult to stain with aniline dyes. Special staining procedures such as Ziehl Nielsen are required. Mycobacteria are also acid fast as they are not easily decolourised with acid alcohol. They are slow growers that take several weeks to appear in primary cultures from clinical specimens (Pfyffer 2015; Egbe *et al.* 2017). When protected from sunlight, mycobacteria are able to survive for months on inanimate objects such as soil or cow dung from which other animals may become infected (Pfyffer 2015). The organisms are not easily killed by freezing or dessication but are heat labile. They are resistant to most chemical disinfectants except those containing phenols, chlorine or alcohol. These properties are important in the spread and control of TB (Pfyffer 2015).

The most significant disease causing mycobacteria are *M. tuberculosis* and *M. bovis* (Hlokwe, Said & Gcebe 2017). The incidence of human TB caused by *M. caprae,* which normally infects goats, has also been reported (Kubica, Rusch-Gerdes & Niemann 2003; Erler *et al.* 2004; Rodríguez *et al.* 2009). Due to the HIV epidemic *M. avium* complex and other environmental mycobacteria known as non-tuberculous mycobacteria (NTMs) can no longer be ignored as they may cause disease in immunocompromised individuals (Rodríguez *et al.* 2009).

1.2 Epidemiology

1.2.1 Distribution and Prevalence

Bovine TB occurs worldwide with the exception of a few countries such as Australia, Sweden, Finland and Denmark which are regarded as bTB-free (Wadhwa & Mahajan 2006). Although bTB eradication programmes are in place in European countries, low endemicity of bTB in wildlife still remains in the USA, Canada, Japan and New Zealand. Some of the wildlife species in these countries maintain the disease serving as a perpetual source of infection and threat to the success of eradication campaigns (El Idrissi & Parker 2012; Fitzgerald & Kaneene 2013). The epidemiology of TB in wildlife is not well understood, and maintaining an elaborate surveillance system in wildlife as in domestic animals is difficult and costly (Pérez-Lago, Navarro & García-de-Viedma 2014).

Bovine TB is still rampant in low income countries such as in Africa due to harsh socio-economic conditions that hinder success of eradication programs (More, Radunz & Glanville 2015). Very few countries in Africa have bTB control measures in place and little or no reporting of the disease is done to the World Organisation for Animal Health (Oficina Internacional de Epizootias – OIE) (Renwick, White & Bengis 2007a). Generally bTB prevalence in cattle is high in Africa with rates as high as 49,8% reported by Munyeme *et al* (2008) in herds in the Kafue basin of Zambia. Lower prevalences of 1.9%, 11.6%, 13,8% and 11.7% have been reported in Tanzania, Ethiopia, Ghana and Nigeria respectively (Tschopp *et al.* 2009).

In South Africa the last report on the prevalence of bTB in commercial herds was in 1995 and it was reported to be less than 1% as cited by Hlokwe, van Helden & Michel (2014).

1.2.2 Hosts

M. bovis has a wide host range which includes domestic animals, wildlife and humans. Bovinidae are the natural domestic host population for *M. bovis* and wildlife can serve as reservoir or spillover hosts.

Reservoir hosts are epidemiologically connected populations in which the pathogen can be maintained and passed on to the natural host population. Reservoir hosts such as the wild boar and African buffalo may also develop the disease (Naranjo *et al.* 2008). Spillover hosts are novel species that have the pathogen transmitted to them by reservoir hosts. These may or may not be able to maintain and transmit disease to other individuals (Palmer 2013).

Globally, different wildlife species have been identified as the reservoir hosts for *M. bovis*. The brushtail possum (*Trichosurus vulpecula*) is considered the reservoir host in New Zealand (Anderson *et al.* 2013), the European badger (*Meles meles*) in Ireland and Great Britain (O'Connor, Haydon & Kao 2012), the wild boar (*Sus scrofa*) in the Iberian Peninsula (Gortazar *et al.* 2012), and the white tailed deer (*Odocoileus virginianus*) in Michigan, USA (Nishi, Shury & Elkin 2006; Corner 2006). In South Africa, the African buffalo (*Syncerus caffer*) is the most significant reservoir host of *M. bovis* (Renwick, White & Bengis 2007b).

M. bovis has also been isolated in other species in South Africa, such as the greater kudu (*Tragelaphus strepsiceros*), lions *(Panthera leo)*, cheetahs *(Acinonyx jubatus)* and baboons *(Papio spp)* (Bengis, Keet & Kriek 2001; Buddle, Wedlock & Denis 2006).

1.2.3 Routes of Transmission

Bovine TB can be transmitted via direct and indirect routes. The direct route involves transmission through sustained and close contact with infected individuals, while the indirect route involves transmission via contaminated materials in the external environment which may include water, feed or equipment (Kwaghe *et al*. 2015).

Direct Transmission

In cattle to cattle transmissions, the main route of transmission is via aerosols, particularly during close contact between animals. Pseudo vertical transmission (transmission via milk) is also very common. An infected dam can pass the infection to her suckling calf and if colostrum and milk is pooled in calf pens large number of calves can become infected this way. Though uncommon, *M. bovis* may also be transmitted vertically from an infected uterus of a cow via the umbilical vessels to the calf (Moda *et al.* 1996). Percutaneous infections are also possible via skin breaks such as wounds or thorn pricks and result in localised lymph node lesions (Kwaghe *et al.* 2015).

Human to cattle and vice versa transmission is also mainly by the respiratory direct route. Abattoir workers are particularly at risk of acquiring lung disease via this route, while ingestion of raw milk and undercooked meat from diseased animals or salvaging animals that would have died from TB poses a risk to the general public particularly in underdeveloped rural areas (Cosivi *et al.* 1998).

Indirect transmission

Indirect routes such as from soil and silage also play an important role in animal to animal transmissions. Body secretions and or excretions including nasal fluids, saliva, urine and faeces voided on soil serve as sources of infection. Mycobacteria are environmentally stable and remain viable and pathogenic in soils for up to six months (Kwaghe *et al.* 2015). Ingestion of soil during grazing and pica due to mineral deficiencies increase the risk of *M. bovis* infection from the environment. Behaviours such as head rubbing and stamping create dust and consequently infectious aerosols (Moda *et al.* 1996). Indirect transmission via human urine contaminated fomites has also been reported (Kwaghe *et al*. 2015).

1.2.4 Risk Factors

A number of risk factors at herd, animal or regional level affect the probability of a herd becoming infected with bTB.

Herd level risk factors

Herd size is a plausible risk factor to bTB transmission. Larger herd sizes with previous infection status have been found to correlate with increased probability of a positive bTB test (Humblet, Boschiroli & Saegerman 2009a; Proaño-Perez *et al.* 2009). Large herd sizes are likely to have large grazing areas with increased probability of interaction with neighbouring herds, and thus promoting cattle to cattle spread. Furthermore, herd size is associated with high turnover rates (introduction of new animals on farm or release of animals into the market) and high stocking density which are in themselves plausible bTB risk factors (Humblet, Boschiroli & Saegerman 2009b). Herd management is also important in the acquisition of infection. Poor management practices such as sourcing cattle from infected herds without testing increases the risk of introducing infection into a bTB-free herd. Other management practices such as crowded housing, use of silage clamps or rotational grazing, poor nutrition in particular mineral deficiencies, test and slaughter biosecurity measures increase or decrease the risk of *M. bovis* transmission. Farms in close proximity to wildlife populations are also at risk of acquiring bTB from reservoir species (Kwaghe *et al*. 2015).

Animal level risk factors

At animal level, immunologically sound and healthy individuals may develop latent TB infections (Kwaghe et al.2015). Increased risk of infection and disease occurs in immunosuppressed individuals or those that have concurrent disease (O'Reilly & Daborn 1995b). Increase in age has been associated with bTB incidence. This is probably because TB progresses slowly, thus animals may be exposed to infection at a young age but manifest overt disease or lesions later on in life (Humblet, Boschiroli & Saegerman 2009b). Latency of mycobacteria with reactivation in older animals has also been described as a possible explanation to increased incidence of bTB in older animals. Other animal level risk factors include genetic variation of animals including breed and gender. Studies have shown that *Bos indicus* breeds are more resistant than *Bos taurus* breeds (Ameni *et al.* 2007). The risk of bTB is attributable to 20% heritable genetic components and 80% to non-genetic components in the environment for example nutrition, husbandry, type and extent of pathogen exposure (Kwaghe *et al*. 2015).

Regional level risk factors

Mammalian species richness (MSR) refers to abundance of biodiversity in an ecosystem and has been noted to have a dilution effect on the spread of bTB (Humblet, Boschiroli & Saegerman 2009b). This is probably due to less competent hosts disrupting *M. bovis* pathways. Emergence and reemergence of infectious diseases have also been attributed to a loss of biodiversity as a result of a decrease of this dilution effect (Roche *et al.* 2012). Humblet, Boschiroli & Saegerman (2009b) found that movement of African buffalo over a large area played an important role in bTB transmission. The large areas occupied by these buffalo result in an increased wildlife-domestic animal interface and increased opportunity to transmit disease. Recent creations of Transfrontier Conservation Areas (TFCA), creating regional human- domestic-wildlife interfaces is also a risk factor for bTB infection across the interface if not managed well (Michel *et al.* 2006).

Bovine TB has a slow clinical course, therefore there is a significant risk of infected ‘healthy looking’ animals to spread infection via animal movement. Both international and local trade drive this risk (Fèvre *et al.* 2006). It is therefore imperative to have movement controls coordinated at national and international levels (Skuce, Allen & McDowell 2012). Globalisation and immigration of humans is a risk factor in the spread of bTB. Rodwell and colleagues (2008) reported increased incidence of *M. bovis* in immigrants of Mexican origin in the United States of America.

1.3 Pathogenesis

The severity of lesions or disease process is dependent on factors such as route of infection, dose and virulence of the pathogen. A more rapid course of disease with a severe pathology is associated with high doses of *M. bovis* (Palmer & Waters 2006). Susceptibility of a species also influences the course of disease. Susceptible species such as cattle only need exposure to a few bacilli to acquire infection while in reservoir hosts, higher doses of *M. bovis* would be required to establish pathology (Palmer 2013). Infection via the respiratory route also results in more severe pathology and disease patterns as compared to the oral route. Percutaneous infection results in localised lymph node infection. It follows that different routes are characterised by different patterns and severity of lesions (Palmer & Waters 2006).

When mycobacteria enter mucus membranes or alveoli, bacterial cell wall components are recognised and both innate and acquired immune responses are activated, with cell-mediated immunity (CMI) being most dominant (Chatterjee & Pramanik 2015). Macrophages engulf the bacteria, attracting neutrophils to site of infection. In immunocompetent individuals, T-cells activate macrophages to engulf and eliminate or control the multiplication of the mycobacteria resulting in latent infection. Due to this competent immunologic response few individuals develop active TB (Domingo, Vidal & Marco 2014).

Mycobacteria have evolved mechanisms to evade the immune system and induce chronic lesions. They have a poorly digestable cell wall and favour an intracellular location within host macrophages. In immunocompromised individuals, these immune system evading mechanisms are effective and the mycobacteria survive and multiply within the macrophages (Hunter 2011). The bacilli population increases within the macrophage and eventually burst it leading to extracellular dissemination of bacteria within and outside the host. The macrophages aggregate in an attempt to contain infection, forming granulomas or tubercles. Necrotisation (a mixture of dead cells, blood, and pus) occurs at the centres of these granulomas giving the characteristic appearance referred to as lung tuberculosis (Domingo, Vidal & Marco 2014). The lesions may coalesce to form large areas of TB pneumonia. The tubercles may also break away and spread via the lymphatic and hematogenous route producing extra pulmonary lesions. Extrapulmonary TB sites include the spleen, brain, kidney, bones, skin and lymph nodes (Elkington, Tebruegge & Mansour 2016).

1.4 Pathology

The most common presentation of TB in cattle is the respiratory form. Granulomatous lesions are formed in the bronchial, retropharyngeal and mediastinal lymph nodes. Granulomatous lesions may also be found in the lungs; they may involve the visceral and parietal pleura. Macroscopically they present as yellowish foci of caseous material (Hunter 2011).

The gastrointestinal tract form is the second most common presentation. Nodules and ulcers are observed on the upper alimentary canal, abomasum, small intestine and large intestine. Other forms of TB include miliary TB, uterine, udder and testicular involvement (Cassidy 2006). Miliary TB manifests as numerous small lesions in a number of organs of the body following haematogenous spread, while udder involvement presents as either caseous TB mastitis or TB galactophoritis whereby glandular lobules are replaced by granulomatous tissue that may calcify (Cassidy 2006).

Microscopic granulomas are often well defined with rounded outlines. The centre is characterised by caseous material and several Langerhans giant cells. Epithelioid cells, lymphocytes, plasma cells and fibroblasts are also seen at the outer layers of the granulomas (Hunter 2011). Occasionally tubercles may not show central caseation microscopically.

In immunosuppressed individuals a granulomatous response may not be elicited. In these non-reactive TB forms, sheets of foamy histiocytes are seen packed with mycobacteria that are demonstrable with acid fast stains (Hunter 2011).

1.5 Clinical signs

Early infections are asymptomatic. It may take months to years for clinical signs to manifest and they are non specific. Animals in the early manifestation of disease appear bright and alert but are more sluggish and docile as the disease progresses. There is a gradual loss of body condition, stunted growth and enlarged lymph nodes. Commonly with respiratory involvement a chronic cough may be present with dyspnoea in the terminal stages (Pollock & Neill 2002).

Gastrointestinal involvement is characterised by diarrhoea or constipation. The reproductive tract involvement in females is characterised by irregular estrus, infertility or recurrent abortions and mucopurulent vaginal discharges while in males testicular abcesses are observed. Other clinical signs are dependent on the location of the lesions, lameness with musculoskeletal involvement, circling, blindness and paralysis with involvement of the central nervous system.

1.6 Zoonotic Tuberculosis

M. bovis is often underestimated as a cause of TB in humans and its dangers are perceived as a remote occurrence (Chatterjee & Pramanik 2015). Bovine TB in humans is often subclinical. Clinical signs include lymph node enlargement, coughing, dyspnoea, weakness, anorexia and emaciation. Clinical and pathological presentations are similar to *M. tuberculosis* and are indistinguishable at that level (Katale *et al.* 2012).

Current incidence data do not reflect the true status of zoonotic TB. This is because there is minimal surveillance of TB in low income countries with high TB burden (Müller *et al.* 2013). Data from non-endemic developed countries has been extrapolated to give a global picture of zoonotic TB. This is problematic because it underestimates the significance of the zoonotic TB epidemic and as a result less scientific attention, intervention and resources are allocated to it in relation to other diseases. Reassessment of global TB is required with special attention focused on TB-endemic low income countries with associated high risk factors such as living in close association with animals and drinking raw milk (Olea-Popelka *et al.* 2017).

Underestimation of zoonotic TB also occurs because characterisation of infection to species level is rarely done. The exact figures of cases attributed to *M. bovis* versus *M. tuberculosis* are thus unknown (Carruth *et al.* 2016). Successful control efforts targeted at *M. tuberculosis* have seen its cases decrease while *M. bovis* cases rise. Currently *M. bovis* contributes about 10% of the global human TB burden (Carruth *et al.* 2016). Forty seven percent *of M. bovis* cases present as pulmonary TB while 53% present as extrapulmonary cases. On the contrary, 82% of *M. tuberculosis* infections are pulmonary cases and only 18% are attributed to extrapulmonary presentation. A World Health Organisation (WHO) (2010) report cited a 116,6% increase in extrapulmonary

TB in Tanzania. Given the differences in presentation of *M. bovis* and *M. tuberculosis,* this could suggest an emerging *M. bovis* epidemic (Katale *et al.* 2012). Contrary to this, Tadesse *et al*. (2017) reported that *M. bovis* contribution to the prevalence of extra pulmonary tuberculosis in Ethiopia was minimal. However, in light of a possible emerging *M. bovis* epidemic, current treatment and diagnostic protocols are ineffective for patients suffering from *M. bovis* and not *M. tuberculosis*. Firstly, because *M. bovis* presents mainly in the extrapulmonary form, misdiagnosis and or under diagnosis is common. Collection of extrapulmonary TB samples is also complex e.g collection of lymph node aspirates versus collection of sputum in the pulmonary form. This contributes to delay in initiating treatment (Olea-Popelka *et al.* 2017). Secondly, routine species characterisation is not done, consequently health professionals may not appreciate the importance of *M. bovis* as a cause of human TB. This low awareness could have contributed to the development of drug resistant strains of *M. bovis* detected in the USA and Spain. This was possibly a result of *M. bovis* being intrinsically resistant to pyrazinamide, a first line antibiotic against TB in humans (Carruth *et al.* 2016). Drugs like rifampicin are more effective for *M. bovis* treatment in humans, but without routine species characterisation health professionals routinely give first line antibiotics that may not necessarily be effective in TB caused by *M. bovis* (Olea-Popelka *et al.* 2017). A nine month treatment regime is recommended for *M. bovis* as compared to a six month treatment regime for *M. tuberculosis*. This also adds to patient compliance and drug resistance challenges and decreases successful *M. bovis* treatment outcomes (Carruth *et al.* 2016).

Risk factors associated with zoonotic TB are typically found in underdeveloped and developing countries while negligible in the developed countries. Besides the more obvious risk factors such as drinking raw milk and eating undercooked meat, some traditional practices common in Africa such as sharing dwellings at night with animals and plastering houses and or floors with cow dung or mud increase the risk of transmission (Katale *et al.* 2012). Poor education results in limited to no awareness of TB and its risk factors. African countries are also the most affected by the HIV/AIDS pandemic and zoonotic TB due to disease correlations.

Addressing zoonotic TB in developing countries is not simple. Proper diagnostics and surveillance need sustained financial resources which are not available in these countries. Addressing risk factors is further complicated by resistance due to cultural norms (Olea-Popelka *et al.* 2017). Attempting to change behaviours such as plastering houses with cow dung without giving cheaper or more accessible alternatives would be met with resistance. Suggesting cement for example would not be feasible due to the already improverished state of these communities. A one health approach with international coordinated leadership and sustained funding is needed to achieve global zoonotic TB eradication (Thoen, LoBue & de Kantor 2010).

1.7 Diagnosis

Diagnosis of bTB may be done based on clinical signs, antemortem tests such as skin tests and immunological assays or postmortem pathology. Due to the economic and zoonotic importance of bTB, diagnostic tests have been central to identifying infected animals so as to institute control measures (Alvarez *et al.* 2017). In spite of this, diagnosis still remains a challenge due to limitations with regards to costs of surveillance, sensitivity and specificity of different diagnostic tools.

M. bovis has a wide host range and despite this, antemortem diagnostic tests remain unvalidated in non-bovine species. There is a species bias in availability of diagnostic tests as influenced by industry interest. However, neglecting diagnosis of TB in other domestic animals (goats, sheep and pigs) and wildlife susceptible to *M. bovis* infection may have a negative impact on eradication campaigns (Broughan *et al.* 2013).

1.7.1 Tuberculin skin test

The tuberculin skin test (TST) is the primary screening test recognised by OIE for bTB detection in cattle. The test is based on the immunological events that take place after infection with *M. bovis* (Schiller *et al.* 2010). Cell-mediated immunity is initiated after infection/exposure with *M. bovis*. On subsequent infections memory T-cells formed during the initial challenge move to the site of infection and replicate to control and curb antigen spread.

During the TST, tuberculins, purified protein derivatives (PPDs) prepared from heat killed cultures of *M. bovis (*bovine PPD) and *M.avium* (avian PPD), are injected intradermally in the animal (Pollock 2000). A cell-mediated response is initiated in infected animals. Visually this is seen as an inflammatory reaction on the skin characterised by skin thickening, edema, redness heat and or necrosis of the skin at the tuberculin injection site. Uninfected animals will not ellicit any inflamatory reaction (Ameni *et al.* 2008). Non specific reactions such as hard cold nodules not accompanied by any other signs such as oedema, redness and pain may be encountered in uninfected animals (Department Of Agriculture, Forestry and Fisheries [DAFF] - 2016).

Two variations of the TST exist *viz*, the single intradermal tuberculin test (SIT) and the single intradermal comparative tuberculin test (SICT). In the SIT only bovine PPD is injected intradermally and animals showing a swelling greater than 2 mm after 72 hours are regarded as positive for *M. bovis* exposure in positive herds. In negative herds or suspect herds, a swelling greater than 6 mm is regarded as positive. In the SICT both the bovine and avian PPDs are injected on different areas of the neck. The difference after 72 hours between post-intradermal measurements and pre-intradermal measurements of avian and bovine sites are subtracted from each other and an animal with a difference thereof greater than 4 mm is regarded as positive (Ameni *et al.* 2008).

1.7.2 Interferon-gamma (IFN-y) assay

The IFN-y assay is a commonly used method to test for bTB. During the cell mediated reactions after infection with *M. bovis*, T-cells produce cytokines including IFN-y which is responsible for activation of macrophages (Alvarez *et al.* 2017). The diagnostic potential of IFN-y release assays is based on stimulation of T-cells using antigen cocktails e.g. tuberculin PPD to produce IFN-y. The released IFN-y is then detected by an enzyme-linked immunosorbent assay (ELISA) such as BOVIGAM® Prionics™. Positive reactions are based on the optical readings as determined by the manufacturer. The OIE recognises this as an ancillary test to the TST (OIE 2017). The IFN-y test has a higher sensitivity compared to the skin test because it detects *M. bovis* infected animals as early as 14 days following infection while reactivity to TST develops 3 to 6 weeks post infection (Bezos *et al.* 2014). The disadvantages of the IFN-y assay especially from the perspective of a developing country is the high cost of the laboratory component and that samples have to reach the laboratory within six hours of collection. It also has a lower specificity than the TST thus precludes it from being an initial screening test for surveillance (de la Rua-Domenech *et al.* 2006; Clegg *et al.* 2017).

1.7.3 Serodiagnosis

The humoural response in bTB is complex. Antibodies (Abs) are normally produced in advanced stages of infection and the titres fluctuate significantly (Proaño-Perez *et al.* 2009). Thus, in domestic animals, serodiagnosis is not widely used in the field as a diagnostic tool. The ELISA is the most common serodiagnosis method and a variety of ELISA tests have been developed such as double recognition ELISA (DR ELISA), indirect and competitive ELISA (Casal *et al*, 2017). They are cheap, simple methods that measure *M. bovis* antibody titres. However, they have a low specificity and sensitivity compared to TST (Proaño-Perez *et al.* 2009). In contrast, a study by Casal and co workers (2017) reported that in high bTB prevalence herds, serodiagnosis has a higher sensitivity than cell-mediated based diagnostic techniques. This high sensitivity potentially reduces the time required to remove infected animals from a herd in eradication schemes.

1.7.4 Postmortem diagnosis

Postmortem diagnosis is used universally at abattoirs to detect TB infections and curb transmission to humans (Katale *et al.* 2012). It also serves as a cheap TB surveillance system (Ayele *et al.* 2004). Welby *et al* (2012) simulated in a stochastic model the Belgium surveillance program sensitivity as well as the impact of alternative surveillance protocols. They found that postmortem surveillance (PMS) at abattoirs had a higher sensitivity than the TST and IFN-y assay. The higher sensitivity was probably due to the large sample size at slaughter houses, nonetheless it proved to be the most effective surveillance component of the Belgian TB surveillance program. At national level it was proven that abattoir surveillance was effective in detecting bTB outbreaks that were not detected at purchase by the means of TST testing (Welby *et al.* 2012). However, the efficiency of PMS is highly dependent on the quality of the postmortem exam and examiner.

Gross postmortem examination at slaughter includes inspection and palpation of lung lobes and lymph nodes. These are then sectioned into 2 cm thick slices to detect lesions. Lesions are characteristic yellowish foci of caseous necrosis. Microscopic identification of mycobacteria and bacterial culture may also be done from postmortem tissue samples. Tissues may also be fixed in formalin for histopathology.

Molecular-based techniques, such as polymerase chain reactions (PCR) are also crucial in accurate species identification and molecular-epidemiology investigations of TB (Michel, Müller & van Helden 2010). Analysis of DNA from MTBC isolates is the basis of all molecular methods. Different DNA fingerprinting methods have different purposes for specific applications. These tests recognise genotype families, detect epidemiological links between samples and thus are used in tracing and transmission studies. DNA fingerprinting is also used in early detection and identification of disease outbreaks, providing an early warning system for the national health services (Asebe 2017). Though molecular methods are plausible with high specificity and sensitivity, it is a challenge to perform them from clinical samples. This is because most clinical samples are contaminated with host and other bacterial DNA, complicating bioinformatic analyses. Culture based DNA sequencing is therefore common but has the disadvantage of being a lengthy process (McNerney *et al.* 2016). In developing countries molecular methods need to be performed by expert personnel, who are hard to come by. They are expensive for these financially strained countries. These methods are thus limited to research or academic centres and specialised TB hospitals (Michel *et al.* 2009; Michel, Müller & van Helden 2010; Asebe 2017).

1.8 Control

In May 2014, the WHO launched the '*End TB strategy*' with a goal towards the elimination of TB (www.who.int). A one-health approach to control zoonotic bTB would complement the *'End TB strategy'* (Cheon *et al.* 2016). In line with this strategy the South African government, via the DAFF, has put in place a bTB national scheme which facilitates testing, identification, slaughtering, isolation, prevention of contact and facillitation of information flow with the ultimate goal of eradicating bTB (DAFF, 2016).

Most countries have adopted test and slaughter policies (Etter *et al.* 2006). Some governments have accompanied these test and slaughter policies with compensation schemes. Farmers are often reluctant to slaughter their animals without compensation, thus this serves as an incentive for compliance (Maye *et al.* 2014). However, the vast human and financial resources required have been an impediment to the success of these eradication programs in most developing nations (Etter *et al.* 2006).

Antemortem and postmortem inspections at abattoirs are also effective bTB control measures (FAO, 2012). Trained animal inspectors ensure diseased animals or animal products are not passed for human consumption or animal food production. Other control methods include heat treatments such as pasteurisation or boiling milk and cooking meat to destroy the heat labile mycobacteria (Humblet, Boschiroli & Saegerman 2009a, Tschopp *et al.* 2009, Katale *et al.* 2012). Good hygiene practices such as disinfection of premises, sterilisation of equipment are also important factors in preventing the spread of bTB from food production plants to the general public.

National eradication programs such as the South African TB scheme have been hampered in areas with abundant wildlife as they may serve as reservoirs of infection and reinfection. Separation of wildlife from domestic animals by game fences controls TB transmission. Herd management and good biosecurity, including quarantine and movement controls are central to bTB control especially at the wildlife-domestic animal interface (Corner 2006; Munyeme *et al.* 2010).

Antimicrobial treatment in animals is discouraged due to the danger of continued shedding of the organism, risk of drug resistance development and transference to the human population. Extensive studies using vaccination as a control strategy have been done but results in field trials have not been encouraging as vaccine trials failed to provide protection in the subjects (Vordermeier *et al.* 2016). Prior exposure of calves to mycobacteria prevented or masked development of protective immunity (Buddle, Wedlock & Denis 2006). However, bTB vaccination in cattle may be available in the near future (Van Der Heijden *et al.* 2017).

1.9 Problem Statement

The TST is recognised universally and used for preliminary diagnosis in bTB control programmes (Ayele *et al.* 2004). Other tests include antibody ELISAs and Bovigam®™ (a commercial IFN-γ assay), used as ancillary tests in eradication and control of bTB (Ayele *et al.* 2004). In South Africa the TST is accepted as the standard field test for diagnosis in bTB control programmes. The TST also remains the definitive diagnostic assay for bTB for external cattle trade such as global export. The advantages of the TST and reasons for its extensive use are low costs, relatively better accessibility of tuberculin PPD, history of successful use, and the lack of better alternative methods to detect bTB (Schiller *et al*. 2010). Despite these advantages the skin test has many known limitations, including the difficulties in field administration and interpretation of results, the need for a supplementary visit to measure the skin response and associated non-compliance by farmers, a low degree of standardisation, and imperfection associated with test accuracy (De la Rua-Domenech *et al.* 2006; Schiller *et al.* 2010). As such, its field application appears unpopular among the field veterinarians and their para-veterinary staff.

Eradication of bTB from cattle in some countries has been unexpectedly protracted, and this has raised questions about the effectiveness of skin testing, particularly when the incidence of disease in the population is low or where there is the potential for contact with environmental mycobacteria. Given the above, recent research has focused on developing alternative, innovative, and complementary testing procedures (Pollock *et al.* 2001; Neill, Skuce & Pollock 2005).

It has been shown that in countries with low bTB, disease prevalence or disease-free status, meat inspection is effective as a diagnostic and surveillance tool (Ayele *et al.* 2004). The importance and impact of meat inspection as a diagnostic and preventive tool cannot be overemphasized. In addition to providing epidemiological information on life threatening zoonotic diseases of meat-borne origin, such as bovine TB, brucellosis, and other toxicoinfections (such as salmonellosis), it also ensures standards for hygienic and wholesome meat free from infections and other toxicoses of inorganic sources are met and upheld (Sa'idu *et al.* 2017).

In 2015 Musoke and colleagues*.* performed a bTB surveillance study on cattle in the Mnisi Community, based in the Bushbuckridge District Municipality, Mpumalanga province, South Africa. They found a low prevalence (approx. 0.34%, excluding suspect animals) of bTB in the cattle population. This low prevalence in cattle may justify the use of point-of-slaughter postmortem evaluation in communal areas for diagnosis and surveillance. VanderWaal *et al.* (2017) studied different surveillance strategies such as PMS and targeted testing as alternatives to routine skin testing in low TB prevalence settings. They found that targeted surveillance was more cost effective and reduced sampling effort by 40% without increasing the incidence of bTB.

Currently, in the Mnisi Community in the Bushbuckridge Municipality of Mpumalanga province, the TST surveillance in cattle is minimally applied due to high costs and unwillingness to conduct the difficult TST by technicians (personal communication with professionals). This is not an unusual phenomenon. O'Reilly & Daborn (1995a) have noted that the TST is not always performed as recommended because of management conditions that make it difficult to perform. Furthermore, bTB TST evaluation has focused on cattle; other livestock species such as goats and sheep have been neglected though they are susceptible to *M. bovis* (Napp *et al.* 2013).

In some parts of Africa, bTB detection has depended on slaughter surveillance as the most economical and efficient method for the detection of infected cattle (Shitaye, Tsegaye & Pavlik 2007). Meat inspection at abattoirs is thus considered as a pivotal and the utmost obligatory method for the detection of bTB or other mycobacterial infections (Shitaye, Tsegaye & Pavlik 2007). In view of the foregoing and considering that such PMS systems, which are seen as cheaper and easier to perform, are adapted to rural communities at the point of personal slaughter and not just abattoirs, would it be a more cost effective and efficacious detection method for TB surveillance versus the current bTB TST in the Mnisi Community which has achieved poor compliance? Also, would the veterinarians and para-veterinarians consider this procedure a less laborious and easily adaptable method?

Farmer awareness of bTB is also paramount for the success of the PMS system. Informing State Veterinary services of impending slaughter will rest on the villagers, and compliance will depend on the awareness level of bTB in the community. It is, therefore, important to determine through this study the level of bTB awareness of the community. Disease control is a multi-factorial multi-stakeholder process with cattle owners as an integral part in supporting policy implementation. Ameni & Erkihun (2007) recommend that livestock disease control strategies should be farmer-based, or should provide an element of determining the level of knowledge of the disease of cattle owners since most of the diseases are associated with cattle husbandry systems.

1.9.1 Aims of the study

1. To assess the comparative probable cost between the TST and incentive-based PMS in the Mnisi Community.
2. To assess the acceptability of incentive-based PMS system by the state veterinary officials, the livestock industry and the community.
3. To evaluate the level of bTB awareness in the Mnisi Community.
4. To provide feedback to the community on the outcome of the study and hence increase their bTB awareness.

1.9.2 Benefits arising from study

This study aims to theoretically evaluate the cost benefit of two different surveillance systems (active vs passive) to enable veterinary services to have a platform for choice between feasible economic choice and opportunity cost (cost of foregone alternative) with regards to bTB control. The extension program that may arise from this research will seek to educate the community and raise awareness levels of bTB. Active participation in disease control activities will develop the farmers' interests and assist disease control experts when adopting workable methodologies aimed at controlling livestock diseases such as bTB in diverse farming communities with varying levels of disease perceptions among cattle owners (Munyeme *et al.* 2010). In addition, because the proposed alternative used the bottom-up approach and community-driven initiative, it carried the potential of ease of acceptability.

CHAPTER 2:

Materials and Methods

Ethics Statement

The project was approved by the University of Pretoria Animal Ethics Committee (AEC), (certificate number v116-16) and Human Ethics Committee (certificate number 374/2016). The authors declare no conflict of interest in this research. Consent was obtained, prior to the administration of the questionnaire, from all the farmers and experts who participated in the questionnaires.

2.1 Study Area

The study area is located in the north-eastern border of Bushbuckridge, a municipality in the Mpumalanga province of South Africa. The study area (Figure 2.1), is a rural community covering an area of 461.03 km^2 and has a population of 66 659 people according to a 2001 census (www.statssa.gov.za). It is bordered by private game reserves and the Kruger National Park (KNP). Communal livestock farming is important to the Mnisi Community. Cattle are the most common domestic animals followed by goats, with very few pig farmers and abundant backyard chickens (www.up.ac.za/mnisi-community-programme).

There are about 12 832 cattle and 15 dip tanks in Mnisi by 2013 estimates (Musoke *et al.* 2015). Farmers are assigned dip tanks according to ”*near me*” functionality (nearness to source of veterinary services). The dip tanks serve as service delivery centres for government veterinary services, including dipping, vaccinations, blood and sample collections for disease surveillance among others. It is a meeting place for most livestock farmers and served as a convenient location for the random selection of farmers to complete the questionnaire.

Figure 2.1. Mnisi Tribal Authority area demarcated in orange. Sampled dip tanks are indicated in orange. *Map adapted from Mnisi community programme study map (www.up.ac.za)*

2.2 Pilot study

In order to evaluate the feasibility of the study, two visits were made to the study area prior to the commencement of the research project. During the first visit the research idea was proposed to the various stakeholders. The researcher identified if the study area would be suitable for such a study, familiarised herself with the conditions, met and engaged with the community.

The second visit was used to test the questionnaires, review based on feedback and create cordiality with the community. Interpreters (environmental monitors employed by the University of Pretoria) were trained on how to conduct farmer interviews and minimise bias during the questionnaire test run (Brace 2008). After the trial run, the questionnaires were validated, and adopted.

2.3 Sampling frame, strategy and sample size calculation

The veterinary services in the study area has a farmer and livestock identification system in place. Every farmer is registered and given a 'stock card' which identifies him and the different animal species and numbers he owns. From the stock card records, a total of 1447 livestock farmers divided between 15 diptanks were identified. Using a simple ballot system (randomization), five of the 15 diptanks were selected (Musoke *et al.* 2015) (Figure 2.1).

In the five diptanks, the following parameters were obtained to calculate the sample size:

Population size (for finite population correction factor or fpc)(N): 552

Hypothesized % frequency of outcome factor in the population (p): 50%+/-5

Confidence limits as % of 100(absolute +/- %)(d): 5%

Design effect (for cluster surveys-DEFF): 1

Using the formula: Sample size n = [DEFF*Np(1-p)]/ [(d2/Z21-α/2*(N-1)+p*(1-p)], an estimated 127 respondents were needed at 80% confidence level. By proportional representations, farmers were recruited randomly from the five diptanks. A minimum of 20 and maximum of 30 farmers were selected per diptank and where farmers did not travel to the diptanks, random door to door interviews of livestock owners were done in the same area serviced by the dip tank.

2.4 Questionnaires and data collection

Two semi-structured questionnaires were developed (Thrusfield 2013). All questions were prepared in English and translated to Xitsonga, the *lingua franca* of the community.

The first questionnaire (Annexure 1) was directed towards the farmers in the Mnisi Community to determine their knowledge, attitude, and perceptions of TB in livestock; challenges faced with bringing cattle three days apart for TB surveillance and opinion on an alternative surveillance system based on postmortem.

The second questionnaire (Annexure 2) was an expert opinion survey. This questionnaire was directed at government officials (veterinary directors, state veterinarians and technicians). It was important to get a representative opinion of the acceptability of the proposed postmortem surveillance system. The expert opinion survey was therefore not limited to the study area, with only one veterinarian, but was opened to at least one expert from each of the nine provinces in the country. Questionnaires were administered by email or by telephonic interview. It included open and closed questions, which focused on the current budget for TB skin test surveillance, challenges faced with the surveillance program, opinion on an incentive-based PMS system and a panel of questions on logistic and other issues that support the application and administration of the current programme.

2.5 Data Analysis

2.5.1 Farmers questionnaire

Data were captured, coded, filtered and analysed using Microsoft Excel. Measures of central tendencies (mean and median values of opinion) were obtained from responses, and descriptive statistics were conducted on all data.

2.5.2 Expert opinion questionnaire

Expert opinion responses were subjected to Delphi opinion survey evaluation (Somerville 2008), and selected experts were approached again for a second opinion that harmonised the first set of responses. Economic data for cost of surveillance were comprehensively analysed using SurvCost® software www.cdc.gov/globalhealth/healthprotection/idsr/tools/survcost.html.

2.5.3 *Economic model and cost benefit analysis*

Budget data were obtained from the State Veterinary services. Additional prevailing economic data were retrieved either through field survey or personal interviews with the stakeholders in the industry. Population and other demographic data as well as prevalence of TB in Mnisi, Mpumalanga or South Africa were obtained through literature review and from the website of Statistics South Africa (www.statssa.gov.za). Using budget information from State Veterinary services, all costs (recurrent, operational and capital expenditure) were calculated under different cost heads (Annexure 3, supplementary material). An annual summary of all costs was generated as outputs, and an integrated disease surveillance cost for TB was produced based on field data using the SurvCost® analysis software. Integrated Disease Surveillance and Response System (IDSR) is a system whereby all diseases of interest in an area through passive or active surveillance are reported together using the same human, capital and infrastructure resources already available to the area (Phalkey *et al.* 2013). SurvCost® uses this system to estimate surveillance costs of diseases of interest. Outputs were generated in tables and graphs. Comparative costs and effectiveness were evaluated using the benefit-cost analyses of overall estimated surveillance costs using TST and PMS.

2.6 Feedback to the farmers

One of the objectives of the study was to give feedback to the community. A feedback session was organized at the end of the study after the completion of data analyses. The results of the study were provided as feedback to the farmers in the communities where the study was undertaken.

CHAPTER 3

Results

3.1 Knowledge of TB and attitude survey in the Mnisi Community

A total of 127 respondents were recruited and gave informed consent to participate in this study conducted in the Mnisi Community of Bushbuckridge Municipality of Mpumalanga province. Of the 127 respondents who signified intention to participate, not all continued until the end of the study or provided complete responses. Only 110 respondents provided useful details for statistical analysis giving a response rate of ≈ 87%.

Approximately 64% of the farmers interviewed were male and only 36% were female. Although most of the participants (93.6%) have heard about TB, approximately 80% of the population did not have accurate knowledge of the range of TB hosts and only 20% could correctly describe TB as affecting humans, cattle and small stock. Of the surveyed households (n=110), 22% have had at least a family member diagnosed with TB, and more than half of the participants (54.5%) admitted to herding animals even while coughing (Table 3.1). In addition, about a third (34.5%) of the surveyed population herd cattle and goats together. Eighty-four percent of the population drinks only commercially prepared milk, and 16% consume raw milk at some point. The majority are willing to call veterinary services during slaughter (88.2%) and provide free samples (92.7%). Many of the participants will either consume slaughtered carcasses with families alone (28.2%), share meat within the community (65.5%) or sell directly to the community (6.3%) (Table 3.1).

On presentation of a photo with TB like lesions, 91.7% of participating farmers agreed to have noticed similar lesions before but only 2.7% would ignore and cook while the majority (97.3%) will carry out partial or full rejection of the infected portion. However, when a picture of a more advanced case of the lungs with calcified nodules was shown, not a single individual agreed to ignore the lesion, but 11% and 39% would cut out the infected part/s or organ respectively and 50% will reject the whole carcass. Eighty percent of the respondents said they would bury or burn infected meat while ≈ 20% will either dispose it in a bin or feed the infected meat to the dogs (Table 3.1).

Cattle herding is carried out by various members of the community depending on availability including hired help, the owner, and family or community members. Ninety-seven percent of the farmers take their cattle to the dip tank approximately once a week, but responses on how often they would prefer to dip their cattle or take them to the diptank for treatment and other operations varied from daily to once per annum with some indicating “never” (6% responses) (Table 3.1).

Table 3.1 Mnisi Community TB questionnaire results

Variable	Category	Percentage of participants
Gender	Male	64.2
	Female	35.8
Heard of TB	Yes	93.6
	No	6.4
TB hosts	Don't know	1.8
	Humans only	60.9
	Cattle and goats	2.7
	Cattle and humans	13.6
	Humans, cattle and goats	20.9
TB diagnosed in household	Yes	21.8
	No	78.2
Persons coughing whilst herding cattle	Yes	54.5
	No	45.5
Goats and cattle herded together	Yes	34.5
	No	65.5
Who herds animals in your household	Son, hired help, self, boys in community	Varied responses
Source of milk	commercially prepared milk	83.6
	commercially prepared milk and raw milk	13.6
	raw milk only	2.7
Concerned on consuming diseased animals	Yes	84.4
	No	15.6
Willingness to call vet services	Yes	88.2
	No	11.2
Willingness to offer sample	Free	92.7
	At a fee	6.4
	Not willing	<1
Frequency of coming to dip tank currently	Weekly	97.3
	Everyday	0.9
	Monthly	0.9
	Never	0.9
How often they would like to come to diptank for other procedures excluding dipping	Weekly	44
	Monthly	42
	Yearly	11
	Never	6
Communal slaughtering	Yes	99.1
	No	0.9
Fate of slaughtered meat	Consumed by household	28.2
	Shared with friends, relatives	65.5
	Sold	6.3
Seen TB like lesions before	Yes	91.7
	No	8.3
Measures taken when lightly infected meat seen	Ignore and cook	2.7
	Cut out infected piece	26.3
	Throw out whole organ	32.8
	Throw out whole carcase	38.2
Measures taken when grossly infected meat seen	Ignore and cook	0
	Cut out infected piece	11
	Throw out whole organ	39
	Throw out whole carcass	50

Disposal of infected part	Bin	1.8
	Bury or burn	80
	Feed to dogs	17.3
	Other	0.9
Refrigerators available in household	Yes	99.1
	No	<1

3.2 Expert opinion survey

Ten experts that included directors, state veterinarians and animal health technicians in the South African veterinary services participated in the expert opinion survey. Questionnaires were sent out as emails, followed up with telephone calls and a response rate of 67% was obtained. In view of the sensitivity of budget matters, and stringent rules regarding non-disclosure, it was a challenge to obtain some detailed budget information from technical experts. While some feigned ignorance or referred the researcher to higher authorities, others ignored the question completely. However, detailed information was obtained from two provinces and this formed the basis for comparison and validation of economic data.

Ninety percent of all experts had a bTB surveillance program operational in the areas covered by their operations. Cattle are the most tested animals for bTB (90%) followed by buffaloes (40%), and lastly goats at 20%. The TST is most used as recommended by DAFF for South African Veterinary Services (DAFF, 2016).

The main purposes cited for carrying out TST were as follows: routine surveillance (60%), movement control (30%), eradication (10%), other disease eradication programmes like brucellosis (10%) and for no tangible objective (10%). The main challenges of the TST reported by participants include among others: poor logistics in the implementation of the herd testing program, centralised and red tape in the procurement system for reagents (tuberculin), lack of timeous feedback to central agency (DAFF) and no consistency in frequency of testing. Budget constraints were also reported to result in operational shortages such as transport, staff, medicines and other consumables. Outbreaks of other state-controlled diseases (foot and mouth disease, rabies, and brucellosis) also divert attention from bTB surveillance. Other challenges were: the handling facilities are not always available, low level of second visits compliance, enthusiasm on the part of technicians for retesting appeared lacking, difficulty in following up of positive results (poor animal identification), difficulty in identifying owners, and reluctance to surrender positive animals for slaughter. Control measures such as quarantine and movement restrictions are also difficult to implement in rural settings as farming systems are not organized and farmers don't see any direct benefit of surveillance. Poor knowledge, attitude, perception and awareness of bTB by farmers was also noted as an impediment to implementation of bTB surveillance.

Only 40% of all experts have verifiable prevalence data. In the majority of veterinary service centers animals are tested but the data are not analysed therefore epidemiological tendencies and implications are unknown.

Overall, 90% of all experts suggested that a PMS system is impracticable and not good for adoption. Reasons advanced for opposing the PMS were: it detects infection very late and so may enhance spread, farmers would use their own resources to report pending slaughter and this might result in low compliance, slaughter rates are low, there will be too many homesteads to follow up, attitude of farmers varies and there are illiterate farmers who may not take the strict implementation seriously, manpower and transport would be a challenge, and that some mortalities may not be reported.

The experts agreed that the TST is not being implemented effectively due to above cited challenges, but do not see PMS as a viable and practical alternative.

3.3 Economic model and cost-benefit analysis

Integrated Disease Surveillance and Response System (IDSR) is a system whereby all diseases of interest in an area are covered through passive or active surveillance and are reported together using the same human, capital and infrastructure resources already available to the area (Phalkey *et al.* 2013). It comes with a Microsoft Excel®-based software (SurvCost®) which performs budget analysis for surveillance.

Data on all important diseases are collected and analysed in the same way by the same people, thus this integrated approach results in a cheaper yet effective/rapid detection and response surveillance system (Phalkey *et al.* 2013).

Using SurvCost®, an integrated disease surveillance and response system implemented for TST will cost R1,783, 242 per annum for the Mnisi community with an average cost of R84.33 per animal. Specifically, 35.4% of the cost will be for personnel, 16.0% for office support, 7.5% for transport, 30.9% for test sundries (i.e tuberculin ,syringes, gloves), 0.2% for treatment and 9.9% as capital cost (Table 3.2, Figure 3.1 and 3.2). For a similar programme implemented for PMS, the overall cost will reduce to R1,320,361 per annum with an average cost of R62.44 per animal. In this case, 47.8% of the cost will be for personnel, 21.7% for office support, 15.6% for transport, 1.3% for test sundries, 0.3% for treatment and 13.4% as capital cost (Table 3.2, Figure 3.1 and 3.3).

Table 3.2 SurvCost® cost analysis

Category		Current TST system costs		Proposed PMS system cost	
		All disease surveillance cost (ZAR)	TB IDSR cost (ZAR)	All disease surveillance cost (ZAR)	TB IDSR cost (ZAR)
Recurrent costs	Personnel	1 490 587	630 902	**1 491 956**	**630 902**
	Office	722 274	286 009	722 274	286 009
	Transport	356 375	**134 389**	365 375	**205 896**
	Laboratory /Test sundries	637 397	**551 217**	56 100	**16 830**
	Treatment	49 564	4371	49 564	4 371
Capital costs	Buildings	38 876	5831	38 876	5 831
	Vehicles	365 389	170 522	365 389	170 522
All resources		3 661 831	1 783 242	3 080 534	1 320 361
Cost per animal		173.18	**84.33**	145.69	**62.44**

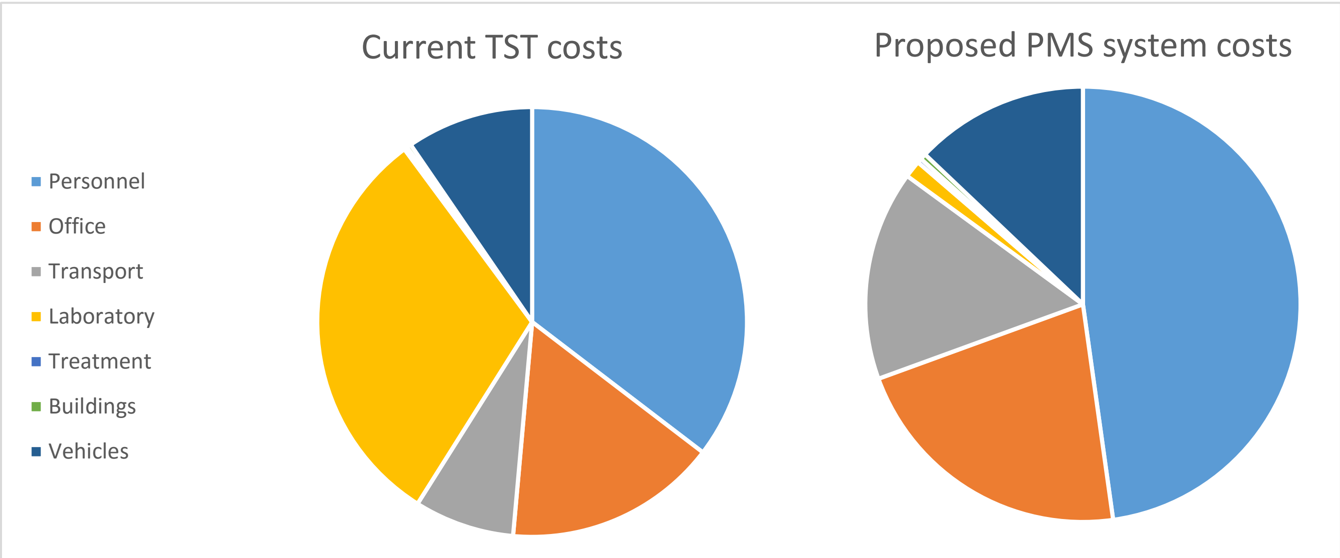


Figure 3.1 Comparison of current TST system costs versus proposed PMS system costs in Mnisi Community, Mpumalanga province as proportion of total IDSR program cost for animal disease surveillance, 2017 estimates

In both graphs, (Figures 3.2 & 3.3), personnel costs were the highest by proportions. In TST surveillance, the costs of tuberculin added significantly to the test sundries cost while in PMS, the test sundries costs decrease proportionately to approximately 1% since there was no need for the skin test. However, the cost for transport increased proportionately in PMS due to costs incurred in door to door communal slaughter postmortem inspections. Overall, in this study, the PMS model appears cheaper than the TST model.

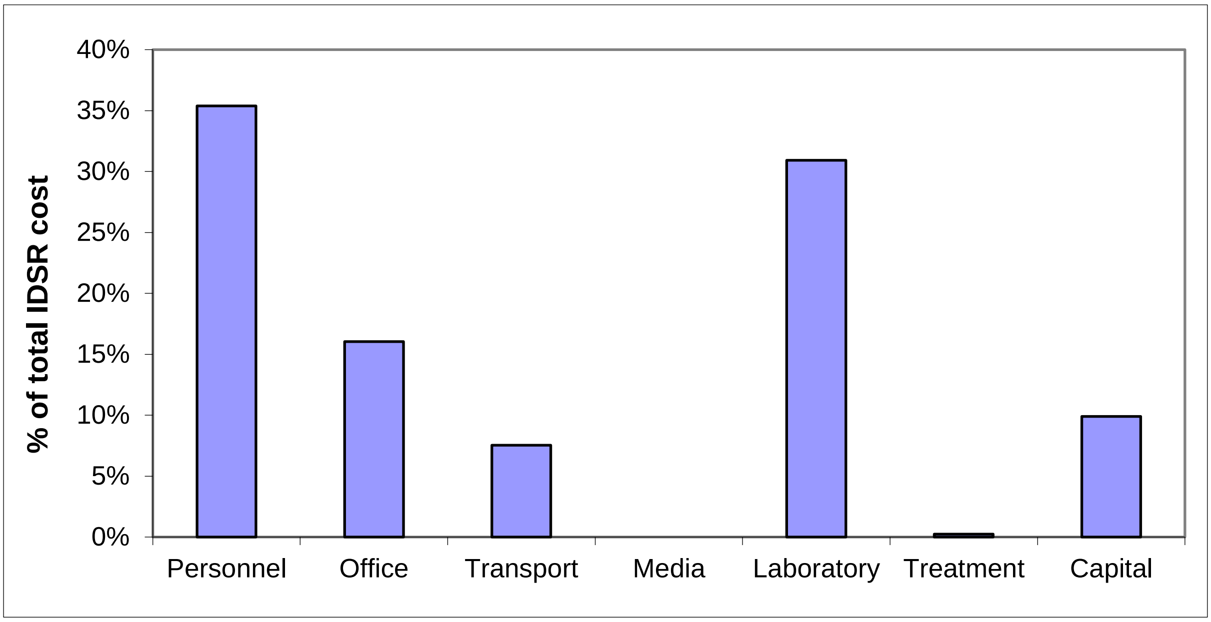


Figure 3.2 TST system costs in Mnisi Community, Mpumalanga province as proportion of total IDSR program cost for animal disease surveillance, 2017 estimates

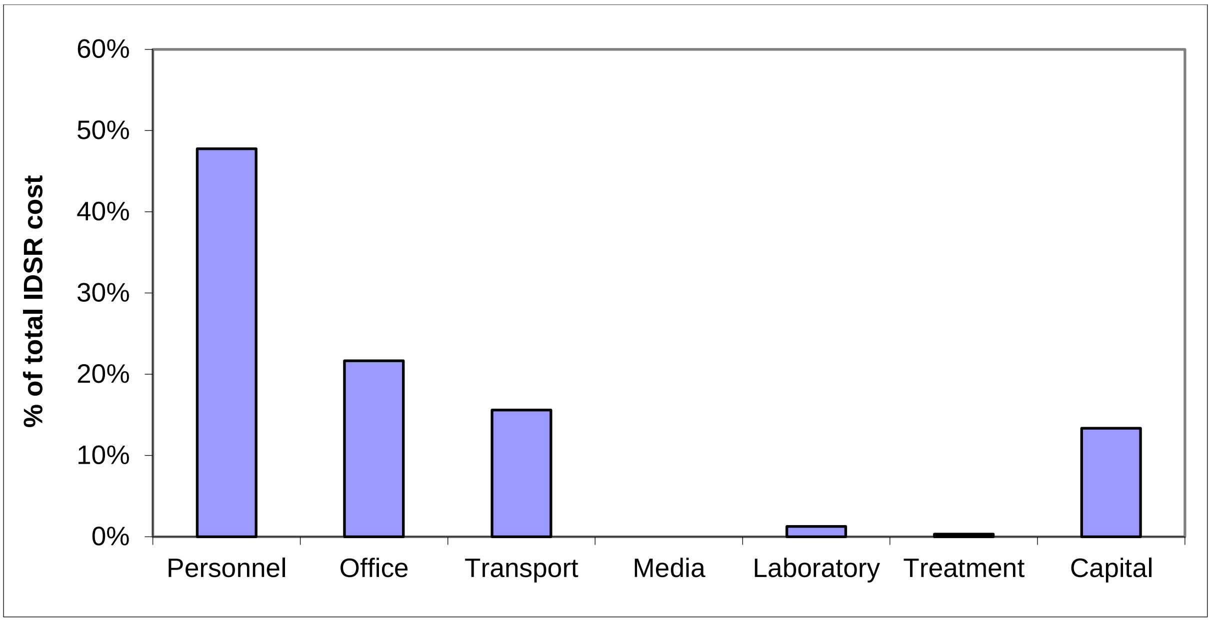


Figure 3.3 PMS system costs in Mnisi Community, Mpumalanga province as proportion of total IDSR program cost for animal disease surveillance, 2017 estimates

The 2017 estimates of cattle population in Mnisi will be 21,145 and at a 3.1% prevalence of bTB in cattle, approximately 656 heads of cattle will be infected (Musoke et al., 2015); at a market value of approximately R12 000 per cow, a hypothetical 100% rejection of infected cattle population will result in losses of R7,872,000. Even, at 50% or 25% rejection rates, assumed losses will amount to R3,936,000 or R1,968,000 respectively (Table 3.3). Through the integration of TST and PMS, the benefit-cost ratio will be 4.41 or 5.96 respectively. While it is evident that conducting the annual surveillance is better in both respects using TST or PMS, the latter (PMS) was at least 1.35 fold economically cost beneficial compared with TST (Table 3.3). Below a 25% rejection rate of the infected animals, the surveillance system becomes non-beneficial for implementation in the Mnisi Community. Similarly, surveillance using TST or PMS based on visceral (lungs and livers) rejection alone cannot justify the investment as inputs far outweigh the expected benefit. (Table 3.3). Additional benefits including the deduction in risk of transmission to other animals and prevention of 339 potential human cases were not quantified in this analysis.

Table 3.3 Cost benefit analysis

s/no.	Items	PMS (in ZAR)	Total (in ZAR)	Benefit/cost (PMS)	TST (in ZAR)	Total (in ZAR)	Benefit/cost (TST)	Comments/Additional benefits	Source(s) of information
1	Cost of surveillance for TB/animal/annum	62,44			84,33				SurvCost® analysis, this work
2	Total number of animal 2017 estimate	21145	1320294		21145	1783158		Cattle population estimate for 2017 was obtained using cattle population in Mnisi for 2013 (n = 12,832) and annual growth rate (n = 13.3) to estimate for year 2014-2017. Prevalence rate of 3.1% was used.	Gaudex, 2014; Musoke et al., 2015;
3	Total number of animal involved at 3.1% prevalence rate of TB	656			656				
4	Mean cost of a cow in rural South Africa	12000			12000			Prevailing market price of adult Nguni cow at auction/rural South Africa (R10,500-R12000).	Market survey, 2017
5	Total cost of animals involved (100% loss)		7872000	**5,96**		7872000	**4,41**	Number of human cases was obtained using total human population estimate for 2015 (n = 80,000), annual growth rate (10.9% over 4 years; 2.75% per annum) and incidence rate of TB in humans for Mpumalannga (0.4%). ***Reduction in risk of transmission to other animals + prevention of 339 human cases are additional benefits not quantified in this analysis***	STATSA, 2012; CEZD, NICD-NHLS, 2015; WHO, 2016
6	Total cost of animals involved (50% loss)		3936000	**2,98**		3936000	**2,21**		
7	Total cost of animals involved (25% loss)		1968000	**1,49**		1968000	**1,10**		
8	Total cost of animals involved (10% loss)		787200	**0,60**		787200	**0,44**		
	Cost of partial rejection alone in Mnisi Community, Mpumalanga/annum								
9	Cost of lungs/kg (R.c)	38			38				Jaja et al. 2016
10	Average weight of lung in a standard cow (kg)	7,8	296,4		7,8	296,4			

11	Total costs for 656 lungs		194438			194438			Musoke et al., 2015;
12	Cost of liver/kg	35			35				
12	Average weight of liver in a standard cow (kg)	6,9	241,5		6,9	241,5			
14	Total costs for 656 livers		158424			158424			Musoke et al., 2015;
15	Total costs (lungs + livers)		352862	**0,27**		352862	**0,20**		Musoke et al., 2015;
Relative ratio of PMS to TST is 1.35, meaning that it is approximately 1.35 times cheaper to institute PMS with relatively equal benefit than to institute TST.									

3.4 Feedback session

A successful feedback session was held at the conclusion of the study (Figures 3.4-3.6). Discussions were held to educate farmers on TB knowledge gaps identified by the study. The farmers expressed appreciation for the good research practice demonstrated whereby researchers come back to communities they conducted their studies and give feedback. However more needs to be done to cultivate trust between researchers and farmers.

Figure 3.4 Mnisi Farmers, researcher and University Of Pretoria students participate in the feedback session.

Figure 3.5 A farmer asks a question.

Figure 3.6 Feedback session with a smaller group of farmers that could not attend main session.

CHAPTER 4

Discussion

4.1 Knowledge of TB and attitude survey in the Mnisi Community

In this survey, majority of the participants were males, a reflection of the demographic structure of the 1,447 livestock farmers. Other studies have similarly confirmed male domination and over-representation/gender bias in the large ruminant sector of the livestock industry including those from Zambia (70%), Zimbabwe (80%) and Canada (91%) (Brook & McLachlan 2006). Mokoele *et al* (2014) have noted that though there are government land reform policies that encourage women to participate in farming, it is still not widely accepted in the rural areas of South Africa. There is a need for a dedicated programme intended to integrate women into the livestock industry in rural South Africa to achieve gender equity.

In addition, a total of 94% of participants were aware of TB. It should be noted that Mpumalanga province has the second highest HIV prevalence rates of all provinces in South Africa at 35.1% (Zuurendonk 2014). The 94% awareness of TB could probably be due to the HIV/AIDS and TB awareness programs initiated by the local health care centres and mass media awareness campaigns, however disease specific knowledge such as the host range was limited because only 21% could correctly identify TB as a disease of cattle, small stock and humans. Since 61% of the participants associated TB with humans only, it can be inferred that many are unaware of its zoonotic potential. This gap creates an inadvertent risk of zoonotic infection to humans who live in close contact and interact daily with cattle and goats and may herd them routinely. A similar report from Ethiopia has confirmed low awareness of TB with 84% of studied participants unaware of transmission routes and risk factors associated with TB (Tschopp *et al.* 2015). Furthermore, more than half of the participants would still herd animals while they were coughing and this remains a significant risk in TB transmission between humans and livestock because a previous study had confirmed that a proportion of the cattle population can be asymptomatic carriers of *M. bovis* (Green 2016). It is known that most causes of cough in humans in rural areas go uninvestigated unless it becomes life threatening. This knowledge and attitude gap will need to be targeted by the health authorities. In addition, human origin *M. tuberculosis* has been isolated in cow's milk indicating probable transmission from humans to cattle (Regassa, Medhin & Ameni 2008). Targeted TB awareness campaigns on knowledge of transmission and risks to promote behavioural changes that discourage herding cattle while coughing, and to reduce occupational risks will become pertinent. Such awareness will also promote health care seeking behaviour (Sreeramareddy, Kumar & Arokiasamy 2013). Studies in India revealed that poor communities that have high TB prevalences with little exposure to mass media also have little knowledge of factors affecting TB risk and transmission (Sreeramareddy, Kumar & Arokiasamy 2013).

In this study, 34% of the respondents herded cattle and goats together, the majority did not. Tschopp *et al.* (2015) have reported similar findings and confirmed low inter-herd risk of disease transmission between goats and cattle as goats are herded around villages separately from cattle that migrate across grazing areas in the pastoralists communities of Ethiopia.

Communal slaughter remains a notable risk factor for the plausible spread of zoonotic TB. A total of 99% of the community members interviewed confirmed to have carried out communal slaughter without supervision of any health authorities. Furthermore, greater than 70% of them will even share the meat with the public while approximately 28% will consume it at home with family members; as such a single case of TB in slaughtered animal or any other rapidly spreading infectious disease like anthrax has a potential of reaching a significant proportion of the community and may exacerbate communal TB spread. Since approximately 92% of the interviewed participants have seen TB-like lesions before, and almost 100% are at risk of infection either through sharing, purchase or home consumption of meat from potentially infected carcasses, incentivised self-reporting of cases may become advantageous in this scenario. It should be noted that communal slaughter may continue for the foreseeable future due to poverty, salvage slaughter, inability of rural farmers to compete fairly in the formal markets and lesser intensity of veterinary service delivery in the rural areas. Such incentivised reporting will come with additional benefits of improved case reporting, reduce burden of zoonoses, and potentially improve human and animal health systems.

The frequency of slaughter per household in this survey is low with most households slaughtering cattle only once per year. This observation may have inadvertently contributed to the low level of reported cases of TB. Whether this report is a true reflection of the human cases of TB is doubtful. Typically, in parts of South Africa and in other developing countries, a reluctance to slaughter large ruminants is linked to wealth assessment based on heads of cattle one owns (Bayer *et al.* 2004, Kunene & Fossey 2006, Green 2016). Though the frequency of slaughter per household is low the overall number of animals slaughtered communally in the whole village per year would be significant to justify a PMS system.

Only 2.7% of participants said they would ignore and cook meat with TB-like lesions. This affirmed earlier findings (Musoke 2016) which stated that most farmers in Mnisi community discard meat with visible abnormalities. It is noteworthy that the majority of the participants understood the dangers of eating meat with apparent lesions. However, of the 38% that responded that they would throw out the whole carcass, whether some of the responses were associated with cognitive bias is unclear, but not unlikely. Of the respondents that will dispose of potentially infected carcasses, 20% opted for discarding in bins or feeding the meat to the dogs. These methods of disposal significantly increase the risk of exposure and transmission of *M. bovis* in the community. Scavengers and carnivores have been known to contract TB from meat sources and from recycled environmental contamination and human sources (Millán *et al.* 2008; Thoen *et al.* 2009; Moravkova *et al.* 2011; Sylvester *et al.* 2017). The risk of acquired infection from drinking infected raw milk in the Mnisi Community is low as only 2.7% of the participants drink raw milk in their households.

Musoke (2016) noted earlier that contrary to popular belief that people in rural areas in developing countries are at risk of contracting *M. bovis* due to drinking unpasteurised milk, majority in the Mnisi Community drink pasteurised commercial milk. We confirmed the same situation in this study. The reasons for this good practice was not ascertained in this study but the ready availability of 'tuckshops or spaza shops' that have sprouted in many villages and which sell pasteurised milk including powdered milk or coffee creamers may be linked to this observation. Noting that the majority of the community members will discard meat with visible lesions and drink pasteurised milk, the risk of contracting TB infection via the ingestion route is low.

Significantly, most of the households (88%) in this survey were willing to call veterinary services to inspect their cattle during communal slaughter, and majority were willing to give biological samples for further testing at no cost while 6% will want payment for a sample to be taken from their animal. This indicates that a PMS system would potentially be well received by the community. Farmers might be more open to PMS because they initiate the slaughter. In TST, animals are slaughtered probably at a time when the farmer is unprepared for such slaughter, hence the degree of resistance and lack of compliance observed. The TST sometimes leads to selling of diseased animals to evade veterinary services or to prevent unintended consequences of reporting zoonotic diseases, for example ostracism and segregation by other members of the community (Mort *et al.* 2005; Kuriansky 2016).

In contrast, experts were of the opinion that PMS is untenable. It is likely that their sentiments are due to cognitive bias wherein professionals are resistant to certain changes and prefer to maintain the status quo. However, it is accepted that significant challenges still exist with PMS as noted by the experts. It will be important to critically evaluate these limitations pointed out by experts with a view to define ways to overcome them.

Farmers were rather concerned by the tick infestation on their cattle and would like their cattle to be dipped every day to control ticks rather than to seek other treatments. Only 44% of the participants maintained the frequent weekly visits as opposed to the 56% who preferred less frequent dip tank visits (monthly 39%, yearly 11%, never 6%). This partially explains the poor compliance with third day return for TB skin test reading reported by the experts in their opinion survey.

Most livestock owners preferred that veterinary services improve in the areas of provision of more crush pens and dip tanks and extensive repair of current infrastructure. Other individual responses, on aspects of improving veterinary services revealed a disconnect and some level of distrust between the community and veterinary services. Such opinions include: concern about policy implementation through a top down approach that lacks consultation, veterinary officials sometimes carry out procedures on their animals without explaining the basis and purpose of the procedures and lack of adequate feedback or no feedback from numerous studies conducted in the community and on their livestock. Cowie *et al.* (2014) have earlier noted that breakdown in relationships between the state authorities and farmers in Britain was due to very different farmer attitudes and views to most effective scientific-based interventions and the practicality of state recommendations of such on bTB biosecurity. This ultimately negatively impacts on implementation and compliance, thus decreasing success that can be achieved in eradication. Robinson (2017) had similarly indicated that the state authorities, as policy makers, enforcers, and advisors to the farmers on many issues are at a conflicting position; despite this, effort must be intensified regularly to create a harmonious relationship and mutual trust among all stakeholders for TB control objectives to be met.

4.2 Expert opinion survey

The experts confirmed that cattle are the most tested animals and the TST is most used test but agreed that the test is under-implemented and not effectively carried out. A review of the implementation strategies and reasons for non-compliance will need to be conducted. It will also be necessary to include all susceptible animals in such a surveillance programme. Furthermore, key challenges have been identified and efforts will need to be engaged to overcome these issues. Because outbreaks of other state-controlled diseases (foot and mouth disease, rabies, and brucellosis) divert attention from bTB surveillance, a comprehensive surveillance that incorporates all the potential endemic diseases may need to be implemented. On the low level of second visit compliance by farmers, intensive awareness campaigns may achieve some degree of success. However, because farmers will prefer PMS, and experts will want a continuation of the TST, a blended approach combining these two surveillance systems may need to be assessed. It will be important for experts to also pay attention to gathering field level data for future epidemiological evaluations. In the present survey, only 40% of the experts have verifiable prevalence data. It should be understood that the diptanks present excellent opportunity for the collation of such epidemiological data.

4.3 Economic modelling and cost benefit analysis

This study provides economic justification for the implementation of surveillance against bTB in the first instance, and showed evidence that PMS is cheaper than the TST.

It is approximately six times and four times more beneficial to perform the PMS and the TST surveillances respectively than to allow the spread of bTB in the Mnisi Community. Mwacalimba, Mumba & Munyeme (2013) have reported that taking into consideration only the monetary value of a cow at point of sale, there is a positive cost benefit to the control of bTB. If a broader approach is considered such as the impact of TB on human health and tourism in TB affected wildlife, there is no doubt that costs associated with TB control are minimal compared to the benefits of eradicating TB (Mwacalimba, Mumba & Munyeme 2013). In this analysis, even for 25% level of losses, it is beneficial to conduct surveillance against TB, however, lower level losses do not justify surveillance in this respect. This may partially explain why countries with low TB prevalences or disease-free status undertake meat inspection only as a cheap alternative for TB surveillance system (Ayele *et al.* 2004).

It is agreed that this benefit-cost analysis has certain limitations because it does not take into account the broader benefits of performing surveillance such as reducing zoonotic disease risk, human loss in quality of life and productivity when sick, loss in animal production, milk and meat yields, loss in tourism and conservation when wildlife species are affected. These may serve as additional reasons to justify surveillance in the Mnisi Community. Mpumalanga has a human TB incidence rate of 0.4% and this translates to 339 human infections in Mnisi Community. The control of TB in animals could potentially prevent a proportion of these human cases (STASTA 2012; CEZD,NICD,NHLS,2015; WHO 2015).

Finally, both models have their own merits and challenges. The PMS model would need increased manpower and transport resources while the TST has not been effective due to difficulty in implementation and compliance. As the TST currently achieves low effectiveness in South Africa, merging the two surveillance systems remains a viable surveillance option. The most effective and cheapest way of controlling zoonotic diseases is to control it at the animal source, and this study may be used as a baseline for future studies to find more effective ways to control bTB in resource-poor or rural communities, and enhance the ‘*End TB’* strategy (Welburn *et al.* 2015).

ANNEXURES

Annexure 1: Mnisi farmers questionnaire

PARTICIPANT'S INFORMATION & INFORMED CONSENT DOCUMENT

Researcher's name: Marange Rudo
Student Number: 16386389
Department of Veterinary Tropical diseases
University of Pretoria

Dear Participant,

A comparison of perceptions of the tuberculin skin test and an incentive postmortem based surveillance system in the Mnisi community, Mpumalanga

I am a masters degree student in Tropical Animal Health in the Department of Veterinary Tropical diseases University of Pretoria. You are invited to volunteer to participate in our research project on a comparison study between tuberculin skin test and an incentive post-mortem based surveillance system in Mnisi community, Bushbuckridge Municipality, Mpumalanga Province, South Africa.

This letter gives information to help you to decide if you want to take part in this study. Before you agree you should fully understand what is involved. If you do not understand the information or have any other questions, do not hesitate to ask us. You should not agree to take part unless you are completely happy about what we expect of you.

The purpose of the study is to assess the comparative probable cost and detection efficiency between skin test and incentive-based post-mortem surveillance in the Mnisi Community, as well as to assess the acceptability of incentive-based post-mortem surveillance system by the state veterinary officials, the livestock industry and the community and to evaluate the level of bTB awareness in Mnisi Community.

We would like you to complete a questionnaire. This may take about 10 minutes. It will be kept in a safe place to ensure confidentiality. Please do not write your name on the questionnaire. This will ensure confidentiality. We will be available to help you with the questionnaire or to fill it in on your behalf.

The Research Ethics Committee of the University of Pretoria, Faculty of Health Sciences, telephone numbers 012 356 3084 / 012 356 3085 granted written approval for this study.

Your participation in this study is voluntary. You can refuse to participate or stop at any time without giving any reason. As you do not write your name on the questionnaire, you give us the information anonymously. Once you have given the questionnaire back to us, you cannot recall your consent. We will not be able to trace your information. Therefore, you will also not be identified as a participant in any publication that comes from this study.

In the event of questions asked, which will cause emotional distress, then the researcher is able to refer you to a competent counselling.

Note: The implication of completing the questionnaire is that informed consent has been obtained from you. Thus any information derived from your form (which will be totally anonymous) may be used for e.g. publication, by the researchers.

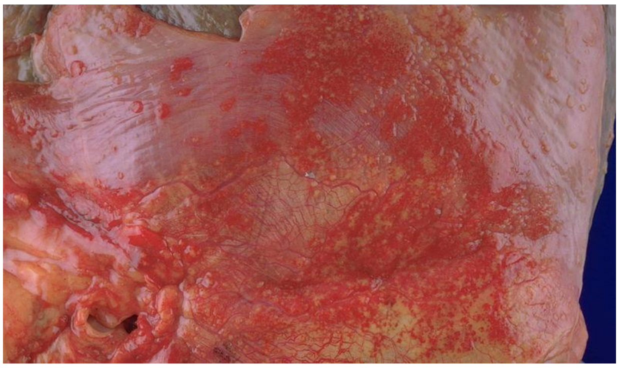
We sincerely appreciate your help.

Yours truly,

Dr Marange Rudo

Mnisi Community TB research questionnaire

1.Have you heard of Tuberculosis (TB)			Yes		No
2. TB is a disease of					
A. I don't know	B. humans	C. cattle and goats	D. cattle and humans only		E. b and c
3. Have you or anyone in your household ever been diagnosed with TB?			Yes		No
4.Who is responsible for livestock herding?					
5.Would this person continue herding cattle if coughing but feeling "strong"?			Yes		No
6.Are cattle and goats herded together by the same person?			Yes		No
7. Source of milk for the household					
A. commercially prepared milk only			B. commercially prepared milk and raw milk from cattle or goats		C. raw milk from cattle or goats
8. How often do you slaughter livestock for personal consumption in a year?					
9. Are you concerned you might be consuming diseased animals unknowingly?			Yes		No
10. Would you call veterinary services to come and inspect your cattle during slaughter?			Yes		No
11. Would you be willing to let them take samples from your cow for free or at a fee?			Free	At a fee	No willing
12. How much would you want to be compensated for a lung lobe of goats and cattle?					

<table>
<tr><td colspan="4">13. How often do you take your cattle to the crush pen for veterinary services?</td><td colspan="2"></td></tr>
<tr><td colspan="4">14. How long does each session last?</td><td colspan="2"></td></tr>
<tr><td colspan="4">15. How often would you prefer to take them?</td><td colspan="2"></td></tr>
<tr><td colspan="4">16. In what areas would you like veterinary services in the area to improve?</td><td colspan="2"></td></tr>
<tr><td colspan="4">17. Does communal slaughtering take place?</td><td>Yes</td><td>No</td></tr>
<tr><td colspan="4">18. Who performs the slaughtering?</td><td colspan="2"></td></tr>
<tr><td colspan="4">19. Who is present during the slaughter?</td><td colspan="2"></td></tr>
<tr><td colspan="2">Men</td><td>Women</td><td>Children</td><td>Men and Women</td><td>Men, women and children</td></tr>
<tr><td colspan="4">20. What do the people involved in the slaughtering process wear?</td><td colspan="2"></td></tr>
<tr><td colspan="4">21. What happens to the meat after slaughter?</td><td colspan="2"></td></tr>
<tr><td>Consumed by the household</td><td colspan="3">Shared with friends, relatives and neighbors</td><td>Sold</td><td>Other? Explain</td></tr>
<tr><td colspan="4">22. Have you ever seen meat with such lesions before? Exihibit A and B</td><td>Yes</td><td>No</td></tr>
<tr><td colspan="6">23. What would you do to meat with such lesions? Exhibit A
</td></tr>
<tr><td>Ignore and cook</td><td colspan="3">Remove infected part</td><td>Throw out whole organs</td><td>Throw out whole carcass.</td></tr>
</table>

24. What would you do to meat with such lesions?

Ignore and cook	Remove infected part	Throw out whole organs	Throw out whole carcass.

25. How do you dispose of infected parts?

Throw in the bin	Bury or burn	Feed the meat to the dogs	Other? Explain?

26. Gender	Female	Male

Annexure 2: Expert Opinion Questionnaire

Expert Opinion Bovine Tuberculosis (bTB) Research Questionnaire

Designation: .... **Date: ....**			
1. Is there a bTB surveillance programme in place for livestock in your area?		YES	NO
2. Which livestock are included in the surveillance programme?			
Only cattle	Cattle and small livestock (sheep and goats)	Other specify --------------------- - --	
3. Which diagnostic tests are used to measure disease surveillance			
a) comparative skin tests	b) post mortem surveillance at abattoirs	c) both (a) and (b)	d) other
If other, please elaborate			
4. What are the objectives of the current surveillance programme?			
5. Are the objectives being achieved? If not, why?			
6. How often are cattle tested for TB?			

7. How many minutes, on average, does it take in handling one animal during movement through the kraal, the line and injection to the exit during the skin testing operation?
8. What are the challenges you face with the current TB surveillance program especially the TB skin test in communal areas?

9. Do you have data on bTB levels in cattle (prevalence) before and after the current surveillance program?				
10. In communal areas do you think the proposed post-mortem surveillance system would achieve the same objective as the TB skin test?				
11. What is the estimated budget for TB surveillance in this community?				
Man hours per 100 cattle	Transport	Avian & Bovine Tuberculins	Miscellaneous costs (consumables, e.g. needles, syringes, gloves, calipers)	Estimated total
12. Are there control measures in place to control bTB in livestock? Please elaborate.				

Annexure 3: Supplementary material, surv cost input data

Personnel Data

Service Unit	**Personnel Category**	Duration of work (number of months per year)	Gross salary per month (local currency)	Incentives and over-time pay per annum	Personnel Time (%) All disease surveillance activities	Personnel Time (%) IDSR as proportion of all surveillance activities
Administration	Chief Director	12	150,367	15,875	2%	25%
	Director	12	132,969	10,250	3%	30%
	Chief Administrator	12	124,120	7,508	5%	15%
Surveillance	State vet	12	53649	6481	75%	50%
	Co AHT	12	38188	5497	75%	65%
	General assistants	12	15958	2830	70%	20%
	Animal Health Tech	12	30996	5497	70%	20%
Support Staff	Accountant	12	52,756	2,850	1%	1%
	Driver	12	30,300	5,275	25%	50%
				Average personnel time	**36%**	**31%**

Office Operating Data

Office Supplies	Total quantity used	Unit cost	Total cost	Quantity used for all disease surveillance activities	Quantity used (%) All disease surveillance activities	Quantity used (%) IDSR as proportion of all surveillance activities
Stationeries	0	0	58 000	50%	30%	30%

Utilities		Number of months	Monthly cost	Total cost	Facility use (%) All disease surveillance activities	Facility use (%) IDSR as proportion of all surveillance activities
Communication		12	7179		75%	40%

Building and Equipment Maintenance		Number of months	Monthly cost	Total cost	Facility and equipment use (%) All disease surveillance activities	Facility and equipment use (%) IDSR as proportion of all surveillance activities
All maintenance		12	51395	616750	20%	40%

Building and Equipment Rental		Duration or No. units	Unit charge	Total cost	Facility and equipment use (%)	
					All disease surveillance activities	IDSR as proportion of all surveillance activities
Rented Building space		12	52892	634704	25%	40%

Transport Operating Data

Program Vehicles	Fuel used (liter)	Total cost of fuel used	Maintenance & reparation cost	Taxes & Insurances & Fees	Vehicle use-time (%)	
					All disease surveillance activities	IDSR as proportion of all surveillance activities
Vehicle A	3,475	50353	21,295	6873	85%	50%
Vehicle B	1,254	18170	15,108	7482	90%	45%
Vehicle C	875	12679	6,750	2,460	75%	45%
Vehicle D	625	9056	4,580	3,057	50%	35%

Rent Vehicles	Duration of rent (days per year)	Daily depreciation charge	Cost of fuel used	Miscellaneous cost	Vehicle use-time (%)	
					All disease surveillance activities	IDSR as proportion of all surveillance activities
Rent vehicle 1	264	264	30000	2,560	95%	50%

Traveler	Duration of travel (days per year)	Daily per diem	Total travel fare	Miscellaneous expenses	Vehicle use-time (%)	
					All disease surveillance activities	IDSR as proportion of all surveillance activities
State vet	45	1175	18000	7200	75%	10%
Co Aht	21	976	12000	6000	85%	35%
Animal Health Technician	21	976	12000	6000	80%	25%
General Assistants	10	976	12000	6000	45%	5%

Laboratory Operating Data

Test sundries Materials	Total quantity used	Unit cost	Total cost	Quantity used for all disease surveillance activities	Quantity used (%)	
					All disease surveillance activities	IDSR as proportion of all surveillance activities
Gloves, syringes consumables			66 000		85%	50%
Laboratory Reagents	Total quantity	Unit cost	Total cost	Quantity used for all disease surveillance activities	Quantity used (%)	
					All disease surveillance activities	IDSR as proportion of all surveillance activities
Testing cost/animal	21145	15	314215	21145	85%	90%

Patient Treatment Data

Item	Total quantity	Unit cost	Total cost	Quantity used for all disease surveillance activities	Quantity used (%)	
					All disease surveillance activities	IDSR as proportion of all surveillance activities
Antimicrobials						
Antibiotics	155500	1	155500	10893	20%	5%
Anticoccidials	3,000	5	13765	1,500	5%	30%

Capital Investment Data

Building infrastructure	Acquisition year	Purchase price	Expected useful life year	Facility and equipment use (%)	
				All disease surveillance activities	IDSR as proportion of all surveillance activities
B1	1996	2,589,340	50	10%	15%

Program Vehicles					
Vehicle A	2012	480900	5	85%	50%
Vehicle B	2013	506000	5	90%	45%
Vehicle C	2014	480900	5	75%	45%

Annexure 4: Ethics Approval Documents

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 22 May 2002 and Expires 28 August 2018.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 22/04/2017.

UNIVERSITEIT VAN PRETORIA
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Faculty of Health Sciences Research Ethics Committee

24/11/2016

Approval Certificate
New Application

Ethics Reference No.: 374/2016

Title: A Comparison study between tuberculosis skin test and an incentive post-mortem based surveillance system in Mnisi community, Bushbuckridge Municipality, Mpumalanga Province, South Africa

Dear Rudo Marange

The **New Application** as supported by documents specified in your cover letter dated 14/11/2016 for your research received on the 14/11/2016, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 23/11/2016.

Please note the following about your ethics approval:

- Ethics Approval is valid for 2 years
- Please remember to use your protocol number (**374/2016**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:

- The ethics approval is conditional on the receipt of **6 monthly written Progress Reports**, and
- 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

Dr R Sommers; MBChB; MMed (Int); MPharMed,PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

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Animal Ethics Committee

Extension No. 1

PROJECT TITLE	**A cost-benefit analysis and comparison study between tuberculosis skin test and an incentive post-mortem based surveillance system in Mnisi community, Bushbuckridge Municipality, Mpumalanga Province, South Africa**
PROJECT NUMBER	**V116-16**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. R Marange**

STUDENT NUMBER (where applicable)	**UP_163 863 89**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES	**n/a**	
NUMBER OF SAMPLES	**n/a**	
Approval period to use animals for research/testing purposes		**January 2017-January 2018**
SUPERVISOR	**Dr. D Morar-Leather**	

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	Date 2 February 2017
CHAIRMAN: UP Animal Ethics Committee	Signature

S4285-15

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