

**Investigation of known risk factors for bovine  
tuberculosis in Namibian cattle**

By

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in the Department of Veterinary Tropical Diseases  
Faculty of Veterinary Science  
University of Pretoria

Supervisor

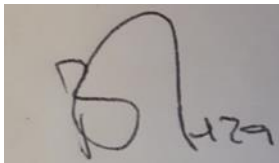
Professor Anita Michel

2020

## DECLARATION

I, Blessing Filda Muza, declare that this dissertation hereby presented to the University of Pretoria for the Master of Veterinary Science degree is my own work and I have not presented it for any degree or award in any other university. All secondary material used was acknowledged and referenced as required by the University of Pretoria.

This research project was approved by the Ministry of Health and Social Services, Research Division of Namibia on the 15<sup>th</sup> of August 2019, the Animal Ethics Committee of the University of Pretoria on the 11<sup>th</sup> of October 2019, the Faculty of Humanities, University of Pretoria on the 12<sup>th</sup> of March 2020 and the Faculty of Veterinary Science Research Ethics Committee on the 4<sup>th</sup> of November 2020.

A handwritten signature in black ink, appearing to read 'B Muza', is shown within a rectangular frame.

**Blessing Filda Muza**

**Date: 04 /11/2020**

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## LIST OF ABBREVIATIONS

BTB(Bovine Tuberculosis)

TB (Tuberculosis)

WHO (World Health Organization)

FAO (Food and Agriculture Organization)

OIE (Office Internationale Epizootis) World Animal Health organization

*M.bovis (Mycobacterium bovis)*

*M. tuberculosis (Mycobacterium tuberculosis)*

UP (University of Pretoria)

IFN- $\gamma$  (Interferon gamma)

HACCP (Hazard Analysis Critical Control Point)

ISO (International Organization for Standardization)

Km<sup>2</sup> (square kilometers)

EU (European Union)

FMD (Foot and Mouth Disease)

UK(United Kingdom)

BCG (Bacillus Calmette-Guérin)

ELISA (Enzyme Linked Immunosorbent Assay)

ESAT-6 (Early Secreted Antigen 6 kilodaltons)

CPF10 (Culture Filtrate Protein)

EFSA (The European Food Safety Authority)

# DISSERTATION SUMMARY

## INVESTIGATION OF KNOWN RISK FACTORS FOR BOVINE TUBERCULOSIS IN NAMIBIAN CATTLE

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Bovine tuberculosis (BTB) is a chronic disease primarily affecting cattle that is caused by a bacterium called *Mycobacterium bovis*. Though less transmissible in nature, *M. bovis* is highly potentiated for transmission between animals by its ability to cause chronic disease. The main mode of transmission of *M.bovis* is through aerosols, however, contaminated pastures and water sources serve as other modes of transmission. BTB is an economically important disease that leads to loss in production in cattle and is of public health concern as it is a zoonosis. There are many risk factors of exposure to BTB in cattle, whilst most of them can be controlled, wildlife contact is a risk factor that remains a persistent challenge in most countries. This project was performed in the 10 cattle farming regions of Namibia to investigate and qualitatively describe the BTB risk factors and their relative importance in Namibian cattle herds.

A questionnaire was designed, translated into the main local languages and a pilot study done before commencement of project. The questionnaire consisted of 23 questions which investigated risk factors of BTB in cattle. The questions were in 3 main sections, namely farm demographics, farming practices and disease awareness. The questionnaires were distributed by hand and by electronic mail to farmer participants. The participating farmers were from Omusati, Kavango, Otjozondjupa, Omaheke, Zambezi, Oshana, Ohangwena, Khomas, Oshikoto and Kunene regions. The results were collected, compiled manually and electronically, qualitatively analyzed and presented using descriptive statistics. A total of 254 farmers participated in the questionnaire survey, of these, 178 were communal and 72 were commercial farmers. Proximity to the border was found to be a high risk factor leading to possible interactions between cattle of unknown BTB status across national boundaries, only 18.3% of farmers indicated to being less than 20 km from the nearest border. Since the risk

of BTB transmission between cattle increases with large herd sizes, this factor was found to be of low risk as 52.7% of farmers had a small herd size of less than 20 cattle. Wildlife species that were observed sharing resources with livestock were warthogs, kudus, mongooses, meerkats, wildebeest, elephants, giraffes and antelopes. Wildlife contact with cattle was the most prevalent risk factor (49.6%), with 27.8% and 33.5% of farmers' cattle sharing grazing and water sources with wildlife respectively, 26.4% of commercial farmers confirmed owning wildlife. Distance travelled by cattle in search of grazing was found to be a medium risk as 19.3% of farmers had their cattle moving for at least 10 km, therefore likely to encounter other herds and or wildlife along the way. Within the previous year, there had been high risk factors of exposure to BTB between cattle. The participants with knowledge of BTB were only 39.7% and only 39.5% knew that it is a zoonosis, which poses a risk of possible exposure to disease out of ignorance. Quarantine of cattle was only done by 22.3% of the farmers, mostly in Khomas region, and only 10% of farmers had their meat for home consumption inspected. The low level of cattle quarantine and BTB testing were found to be very significant risk factors of exposure to BTB as diseased cattle were potentially being introduced into healthy herds. Most farmers (62.7%) slaughtered cattle for meat, only 30% of farmers confirmed taking abnormal carcasses to the veterinarian for post-mortem examination. The absence of meat inspection and failure to take abnormal carcasses for meat inspection were significant risk factors for human exposure, since if BTB lesions were present, they could remain unidentified. Milk was found to be a low risk factor of exposure to zoonotic BTB as 67% of farmers confirmed not consuming milk. A total of 12% of farmers confirmed having had a history of human TB in their households, which was a significant finding taking into consideration the possibility of zoonotic *M. bovis*. There was sufficient evidence from the results that although other risk factors of BTB are present, wildlife is the most threatening common risk factor posing the greatest challenge in Namibia.

# 1. CHAPTER ONE

## GENERAL INTRODUCTION

### 1.1 Background

Bovine tuberculosis (BTB) is a chronic disease primarily affecting cattle that is caused by a gram negative acid fast bacterium called *Mycobacterium bovis*. Though poorly transmissible in nature, *M. bovis* is highly potentiated for transmission because of its chronicity. It is mainly transmitted through inhalation of air droplets, however, oral transmission occurs if cattle graze on infected saliva contaminated pastures (Fine et al., 2011). Additionally, contaminated water sources are a source of infection (Fine et al., 2011).

BTB is an economically important disease that leads to loss in production in cattle. Moreover, its presence can lead to trade restrictions which lead to loss in export earnings. The disease is a zoonosis which can lead to a chronic debilitating disease in humans largely indistinguishable from human tuberculosis caused by *Mycobacterium tuberculosis* and is thus of public health concern (Kahler, 2015). There are many risk factors for exposure to BTB, whilst most of them can be controlled, wildlife contact is a risk factor that makes eradication nearly impossible in most countries (Sergeant et al., 2017).

### 1.2 Problem statement

Freedom status from Bovine Tuberculosis (BTB) is of invaluable importance to trade. Post BTB eradication, Namibia has been reporting freedom from BTB to the OIE based on surveillance data. Although BTB testing is done, it is limited to imported animals, weaned beef cattle that are exported to South Africa and dairy cattle herds (OIE, 2018; Chitate et al., 2019). Risk factors of exposure to BTB are probably in existence in Namibia though they have not been officially investigated and evaluated. These factors include presence of wildlife, movement of cattle across national boundaries into countries without freedom from BTB and local interactions between cattle herds of unknown BTB status. It has been shown worldwide that the presence of wildlife is a risk factor largely because the control of the disease is very difficult in wild animals (Fox et al., 2018), so attention must be given to livestock-wildlife interfaces in existence when ascertaining freedom. The BTB status in Namibian wildlife is unknown therefore the presence of wildlife in some areas especially

the known reservoir species where cattle for export are reared may threaten freedom. Cattle reared in close proximity with wildlife may be susceptible to spillover infections that bring freedom into question. The same spoligotype and genotype of *M. bovis* was found in cattle adjacent to and in wildlife in the Greater Kruger National Park, which strongly suggests possibility of spillover of infection from wildlife species (Musoke et al., 2015). The presence of a BTB reservoir or spillover hosts capable of transmitting the disease thus increasing the risk to cattle may act as pointers to high risk areas for the presence of BTB. Secondly, the possibility of introduction of new animals whose BTB status is unknown into herds which may potentially be infected also poses a risk for susceptible cattle. Presumed BTB free cattle may have interactions with other cattle of unknown status creating a possibility for transmission between cattle populations. Existence of both legal and illegal cattle movement across regional or national or international boundaries create possibilities of disease transmission to vast herds with serious disease consequences. Unfortunately, compliance with laws is not achieved totally. Smuggling and theft of cattle across national boundaries even done on a small scale can represent another serious portal of infection. Another loophole that has been discovered is a lack of cattle movement control by veterinary services in the north particularly close to the Angolan border, which may allow passage of infected cattle into Namibia compromising the BTB freedom (OIE, 2011). Since BTB was formerly present in Namibia, pockets of infection may have persisted but remained undiagnosed. This has been found in South Africa whereby undiagnosed wildlife have potentially played an additional role in spreading disease since their movement except for buffalo is not controlled (Hlokwe et al., 2014). Taking these factors into consideration, BTB risk factors have to be investigated in Namibia when ascertaining freedom from the disease.

### **1.3 Research questions**

Which BTB risk factors reported in the scientific literature are present in Namibian cattle, their significance and how are they distributed in Namibia within the farming types?

#### **1.4 Objective**

To qualitatively describe the BTB risk factors for BTB in the cattle population in Namibia (across farming systems)

#### **1.5 Benefits from the Research**

Farmer awareness to BTB and its risk factors will be raised as this disease is of economic importance due to its chronic nature. Knowing risk factors will allow a risk based surveillance strategy which is more cost effective for the Ministry of Agriculture, Water and Forestry. If need be, depending on outcome of research recommendations can be made for better control strategies and policies to maintain the BTB freedom status in Namibia thus benefiting the whole nation. A Master of Science dissertation and degree for postgraduate studies will be achieved.

## **2. CHAPTER TWO**

### **LITERATURE REVIEW**

#### **2.1 Characteristics of *Mycobacterium bovis***

Bovine tuberculosis (BTB) is a chronic disease primarily affecting cattle but with the ability to jump between species. It is caused by a gram positive, aerobic slow growing acid fast bacterium called *Mycobacterium bovis*. The bacterium's higher classification is genus *Mycobacterium*, phylum Actinobacteria, order Actinomycetales and is within the family Mycobacteriaceae. Morphologically, *M. bovis* appears in stained tissues as thin rods which are straight, curved or club shaped, 1-4 micrometres long and 0.2-0.5 micrometres wide, however, in culture *M. bovis* is observed as cocci or bacilli.

#### **2.2 The epidemiology of Bovine Tuberculosis**

##### **2.2.1 Pathogenesis of *M. bovis***

Individual animal level of response to infection varies with factors that contribute to risk of infection and severity of disease identified as age, breed, animal genetics, production system practiced (Broughan et al., 2016; Downs et al., 2018) and body condition score of animal. At herd level, herd size, type of housing, close contact between herds and contact with wildlife were found to be significant risk factors with a positive correlation to infection. In areas where cattle go out to pasture, it has been found that a large herd size increases the risk of contact with wildlife because the herd is likely to travel far and wide in search of grazing where they are likely to come in contact with wildlife (Dejene et al., 2016).

Incubation period ranges from months to years with acute disease known to occur if there is rapid development and spread of tubercles (FAO, 2012). Diseases which present the same symptoms and lesions as those in BTB include bovine pleuropneumonia, pneumonic pasteurellosis, bovine measles, corynebacteriosis, echinococcosis, lung abscesses or generalized abscessation and bronchopneumonia.

##### **2.2.2 Transmission of *M. bovis***

The main method of *M. bovis* transmission is aerosol although oral transmission is also possible. A large herd size leads to a high concentration of aerosols with an increased encounter of infected and susceptible animals which favours transmission of the pathogen (Dejene et al., 2016). *M. bovis* can remain viable and pathogenic in the environment for

varying time periods depending on the existing environmental conditions. It has been established that the bacterium can survive on hay for about 6 weeks in cold weather and for a few days in summer (Fine, 2011). Survival in water of *M. bovis* ranges from 3 weeks to 2 months depending on the conditions, it has been established that stagnant water sources are infective up to 18 days post infection (Ayele et al., 2004). Its survival in soil was established to be 2 weeks in summer and about 3 months in winter and even much longer if cold dark conditions were provided (Maddock, 1933; Fine et al., 2011). It has also been established that *M. bovis* can stay in natural water sources used by wildlife (Cowie et al., 2016). Therefore saliva, urine and other secretions or excretions from infected animals can be taken into the soil and ingested together with soil as susceptible animals feed (Kwaghe et al., 2015). Cow dung can harbour the bacterium for at least 5 months in winter, 4 months in autumn, and it can survive for 2 months in summer and can live in soil for up to 24 months (Wray, 1975; Fine et al., 2011). Grazing pastures contaminated with saliva from animals infected with *M. bovis* is another important source of infection (Dejene et al., 2016). Cattle, in particular when grazing take up the soil which can constitute 5-10% of the fresh feed intake and 10-15% of the dry matter intake (Kwaghe et al., 2015) potentially making this route an important portal of infection.

### **2.2.3 Wildlife reservoirs**

Despite cattle being considered to be the true hosts of *M. bovis*, it has a very wide host range of mostly warm blooded animals (Kahler, 2015). The disease causing pathogen has been isolated in domestic and non-domestic animals including buffaloes, elands, impalas, lions, bison, raccoons, cheetahs, mongooses, dogs and cats (Anderson et al., 2013). This wide host species range makes *M. bovis* easily transmissible and resilient because of the ever existent possibility of potential reservoirs of infection for domesticated cattle in the wildlife population.

The two categories of hosts within the wildlife populations are the maintenance hosts and the spillover hosts. The maintenance hosts have the ability to be permanently infected with a pathogen and disseminate it to a target population (Haydon et al, 2002). Reservoirs of infection have been found to be bovids specifically buffaloes in Africa, marsupials, particularly possums in New Zealand, cervids, in particular deer and elk in North America, whilst mustelids, in particular badgers are problem animals in Europe (<https://www.tbfacts.org/bovine-tb/>). While the maintenance hosts can harbor and disseminate infection, spillover hosts can only disseminate infection to susceptible host populations (Humblet et al., 2009). A multiple host pathogen system has also been described whereby a number or group of species can act as a maintenance or spill over host

community (Renwick et al., 2007). Furthermore, the host range for BTB is ever increasing, making pathogen propagation very possible. This poses a continuous threat as it is a source of infection to cattle and has proven to be a hindrance to BTB eradication in many developed countries, making total BTB eradication nearly impossible (Kwaghe et al., 2015).

Worldwide, the information pertaining to the epidemiology of BTB in wildlife is missing or scant particularly in the developing nations in Latin America, Asia and Africa (Awada et al., 2018). This poses a challenge as wildlife plays a major role in the epidemiology of the disease in cattle (Awada et al., 2018). With this limited information, disease control and surveillance efforts can become misdirected and slow down disease eradication in problem countries. However, badgers, possums and the wild boar have been extensively studied with evidence suggesting contact with cattle perpetuating infection (Fitzgerald and Kaneene, 2012). Evidence has been found in New Zealand that a significant reduction in possums and ferrets in a particular location resulted in a reduction of ferret BTB lesions by 80% for up to 5 years (Caley et al., 1999). Wild boars have been strongly implicated as they have harboured the same BTB genotypes found in cattle and the pathogen has been found present in wild boars even in areas without domestic animals (Martin-Hernando et al., 2007; Naranjo et al., 2008). BTB control strategies in wildlife include reducing the wild animal population, ideally removing the diseased animals and replacing with the disease free, vaccinating wildlife to break the transmission chain to livestock, creation of buffer zones to limit wildlife-livestock contact, good biosecurity practices to eliminate any form of contact between livestock and wildlife and efficient surveillance that can ascertain infection status in wildlife allowing the protection of susceptible domestic animals (<https://www.tbfacts.org/bovine-tb/>).

#### **2.2.4 Animal movement**

Although animal movement is controlled in Namibia within the EU export cattle zone, there is a possibility of animal movements into other farms or areas with less stringent control measures, and these animals may serve as disseminators of infection. Introduction of new animals is another risk factor, particularly if they were not tested for *M. bovis* prior to introduction and not coming from a reputable herd. The work of Reilly and Courtenay demonstrated that the risk of BTB exposure is reduced by introducing less animals and introducing calves and yearlings instead of adult cattle (Reilly and Courtenay, 2007). In Namibia, for any live cattle imports, the law requires the cattle to be coming from BTB free herds and it is mandatory to have cattle tested with the tuberculin tests successively twice not less than 6 weeks and not more than 6 months apart. In addition the law requires the tested animals to be kept in isolation between the two tests and transferred to the new owner within 2 weeks of the last test (Namibia Government Gazette, 2009). Unfortunately,

compliance with laws is not achieved totally, and smuggling and theft of cattle across national boundaries even done on a small scale can represent another serious portal of infection (Mogotsi et al., 2016). It has been demonstrated worldwide that introduction of an infected animal into a disease free herd is a major risk factor for disease introduction (Humblet et al., 2009). Another loophole that has been discovered is a lack of capacity within veterinary services in the north, particularly the Angolan border, which may allow passage of infected cattle into Namibia compromising the BTB freedom status (OIE, 2009).

Given the extensive cattle production system in Namibia whereby animals are allowed to graze on expansive pastures and move over large tracts of land in search for food, interaction can be protracted, allowing for exchange of infection. This can be exacerbated by general neglect by farmers and/or herders towards the upkeep of their animals, as seen in Botswana's efforts in trying to control FMD by preventing straying of cattle (Mogotsi et al., 2016). The fences put across national borders meant to be animal proof are prone to damage particularly by wildlife, which allows cattle to stray across national boundaries and get into contact with other cattle whose BTB status is not known.

### **2.2.5 Tuberculosis as a zoonosis**

O'Reilly and Daborn suggested that the zoonosis of *M. bovis* is caused largely directly by the respiratory route. Indirect transmission is through bedding and hay that has been contaminated with human urine, renal excreters were reported by Huitema in 1969 in the Netherlands and by Schliesser in 1974 in Germany (O'Reilly and Daborn, 1995). *M. bovis* infected humans were the source of infection in 50 cattle herds when a total of 636 tuberculin reactor cattle were identified, of which 497 were confirmed at post mortem as *M. bovis* infected (O'Reilly and Daborn, 1995). Among the 50 humans, 24 were *M. bovis* infected and had urogenital tuberculosis, whereas the rest had mostly pulmonary tuberculosis (O'Reilly and Daborn, 1995). In Germany, *M. bovis* rarely occurs, with most of the cases found to be human to cattle transmission (Ayele et al., 2004). Whilst this route is extremely unlikely in Namibia, there is a high incidence of human tuberculosis particularly the extra-pulmonary form. Since only animal movement is restricted, infected persons may transmit infection to susceptible animals or herds. In a Danish study, immigrant workers were found to be sources of infection for cattle raised intensively (Anderson et al., 2013). However this is probably very remote in Namibia where cattle are mostly raised extensively.

Human transmission of *Mycobacterium tuberculosis* to cattle is of great importance, as cattle act as spillover hosts, however, disease is not as severe as with *M. bovis* (Hlokwe et al., 2017). *M. tuberculosis* transmission to cattle mostly occurs in countries with high numbers of

human TB infections (Romero et al., 2011). In Ethiopia, the main methods of transmission were found to be by aerosol inhalation during close contact with humans and via saliva through spitting of chewed tobacco into cattle mouths with the belief of it enhancing performance (Ameni et al., 2013). In other isolated studies, cattle with *M. tuberculosis* were found to be 6.2% in Algeria, 7.4% in Sudan and up to 27% in Ethiopia (Sulieman, 2002; Ameni et al., 2011).

Coupled to its crippling economic implications, BTB is a common zoonosis worldwide despite the level of development. Transmission to humans is through the respiratory and cutaneous routes, orally through milk and milk products, and meat and meat products particularly undercooked meat. Meat from infected animals, however, has fewer implications as the bacterium is easily destroyed by heat during normal cooking (Walsh et al., 2008). Before the advent of mandatory pasteurization of milk, it was found that a quarter of tuberculosis cases were attributable to *M. bovis* in the developed world and about 15% of all cases of tuberculosis in humans in the developed world up until the end of 1990's were believed to have been due to *M. bovis* (Ashford et al., 2001). Recently, another study has reported a decline in the figure to 2.8% of the human tuberculosis case load being caused by *M. bovis* in Africa (Müller et al., 2013).

In most of Africa and the rest of the developing world the epidemiology of human tuberculosis caused by *M. bovis* and the occurrence of extra pulmonary tuberculosis is unknown (Chen et al., 2009). Generally, the prevalence of BTB in humans is underestimated largely due the lack of diagnostic distinction between *M. bovis* from *M. tuberculosis* infections. Namibia has been reported to have had the fifth highest number of cases of human tuberculosis worldwide in 2007 (WHO, 2009) with *M. bovis* being implicated as the cause of a form of tuberculosis in humans that is characterized by extra-pulmonary lesions (Kahler, 2015). In 2012, WHO recorded 11 145 human tuberculosis cases in Namibia of which 2063 were extra pulmonary (WHO, 2012). This could also indicate the role of *M. bovis* in human caseloads since definitive diagnosis to determine specific pathogens is not made. Since human infection with *M. bovis* is naturally derived from cattle, this acts as a potential pointer to the status of Namibian cattle with regards to *M. bovis*. Meat from infected animals which are slaughtered privately where there are no meat inspection facilities or experts can be a source of human infection particularly in the remote communal areas of Namibia. As Kahler put across in her work, the latter information supports the contention that the absence of BTB outbreaks in Namibia over the last decades cannot rule out the possibility that some cases were undiagnosed or went unreported (Kahler, 2015).

## **2.3 Diagnosis of *M. bovis***

### **2.3.1 Ante-mortem diagnosis of *M. bovis***

Ante-mortem diagnosis of BTB infection in cattle is done using standard detection methods on the basis of cell mediated delayed type hypersensitivity reactions. These tests can diagnose subclinical infection, hence there are used in field surveillance (De la Rua-Domenech et al., 2006). Three variations of the tests are in use, namely the single intradermal comparative tuberculin test, the single intradermal tuberculin test and the caudal fold tests, all which use a purified protein obtained from mycobacterial cultures. The tuberculin is a protein extract from the cell wall of *M. bovis*. When injected into the skin of an infected animal, the sensitized immune response of the animal causes a localized inflammatory reaction typical of a positive test. The tuberculin potency (Downs et al., 2013), type of test and the adherence of testing personnel to the standardized protocol have an influence on the sensitivity and specificity of these tests (Humblet et al., 2011). The BOVIGAM assay is an OIE approved second test used alone or as an ancillary test to the tuberculin skin test based on the production of gamma interferon (Vordermeier et al., 2006). Tuberculin purified protein derivatives are added to whole blood samples and incubated overnight, lymphocytes are stimulated to produce interferon gamma, which is estimated using a sandwich ELISA (OIE, 2015). Consequently, interferon detection correlates with infection as lymphocytes of uninfected cattle do not produce interferon gamma in response to tuberculin antigens. The test has a better sensitivity but equal or lower specificity compared to the intradermal skin tests (Pucken et al., 2017). However, the single intradermal comparative cervical test is prescribed by OIE for the ante-mortem diagnosis of BTB in cattle. The test involves measuring the skin thickness in the region of the neck and then injecting bovine tuberculin and avian tuberculin intradermally. The skin thickness is then measured 72 hours later for any subsequent swelling. In addition to the measurement, there are clinical signs to be taken into consideration which are indicative of a positive reaction. Visual appraisal is done to identify necrosis, exudation and swelling. Palpation is done to ascertain consistency of swelling, presence of oedema, enlarged peripheral lymph nodes, heat, pain and adhesions between the skin and subcutaneous tissues. Cattle with advanced disease may start to show symptoms such as coughing, pneumonia, fever, shivering, ruffled hair coat, listlessness and decreased milk production 24 to 48 hours after the test.

The advantage of the comparative intradermal test is that it can distinguish animals truly reactive to *M. bovis* from those that are previously sensitized to other mycobacterium antigens. Molecular assays are not validated, therefore are currently not in routine use except for research purposes (Kahler, 2015).

### **2.3.2 Postmortem diagnosis of *M. bovis***

After death or slaughter, BTB diagnosis is made at post mortem by inspection for lesions consistent with the disease. Worldwide, all cattle slaughtered for beef for human consumption through formal markets go through mandatory standard meat inspection (Downs et al., 2018). Confirmation of *M. bovis* is made through histopathology of lesions containing the bacterium or bacterial culture, which remains the gold standard for diagnosis with its only limitation being its practicality since it can only be done at postmortem.

### **2.4 Pathological changes caused by *M. bovis***

The main system affected by *M. bovis* is the respiratory system and its accompanying lymph nodes, therefore, that is where most lesions are concentrated, which are nodular granulomas commonly referred to as tubercles. On visual appraisal these tubercles are cream to yellowish in colour, caseous or calcified though they can occasionally be purulent. However, in some instances they can be very small such that they cannot be seen with an unaided eye (Chambers et al., 2018). Tubercles often have a dry caseous centre and are enclosed in a fibrous connective tissue capsule (Chambers et al., 2018).

Thus the most common findings made at postmortem are these tubercles of varying sizes within the lungs and swollen lymph nodes in apparently healthy animals being slaughtered for human consumption, since most cases are subclinical. BTB is a chronic disease insidious in nature causing coughing, dyspnoea, severe emaciation and debilitation of cattle, symptoms which are seen at the end stage of the disease.

The location of the BTB lesions are pointers to the initial route of infection, thoracic lesions being attributed to aerosol transmission and gastrointestinal being attributable to oral infection (Buddle et al., 2002).

BTB is a disease of utmost socioeconomic importance, causing overall reduced production in cattle, great financial losses through carcass condemnation and the brunt of costs of surveillance and disease control and export restrictions. It causes chronic pain and suffering in animals because of its insidious nature, which makes prompt diagnosis very difficult.

## **2.5 Prevention and control of Bovine Tuberculosis**

### **2.5.1 Vaccination for *M. bovis***

Vaccination for *M. bovis* is still a challenge as there are currently no registered vaccines against BTB for use in animals. Unlike in human medicine where vaccination is routinely done, experimental animal vaccines under research have a variable efficacy and therefore still need further development to serve their intended purpose. For instance, a BCG vaccine in cattle would result in sensitization, which interferes with diagnostic intradermal tests. This necessitates a complex blood test that detects IFN- $\gamma$  to other antigens in *M. bovis* (ESAT-6 and CPF10) not present in the BCG vaccine to differentiate the vaccinated from unvaccinated (Vordermeier et al., 2006). In spite of this, BCG formulations are promising and likely to be registered, so far, a BCG vaccine is being used in the United Kingdom in badgers to control BTB (Rutten and Michel, 2014).

### **2.5.2 Control of *M. bovis***

Successful control of the disease has been achieved in most developed countries and regions of the world such as Australia, Europe and New Zealand by regulating animal movements and using the test and slaughter policy, but it still remains a big challenge in the least developed regions in Africa, Asia and South America (Friyantha, 2008). The test and slaughter policy relies on abattoir, periodic herd level and risk based surveillance which focuses on herds exposed to most risks and therefore likely to be infected (Napp et al., 2019). During slaughter surveillance, if an infected carcass is identified, the herd of origin is traced, tested and positive reactors are slaughtered and the animal owner reimbursed. However, this has remained impractical in heavily infected countries which necessitates the slaughter of large numbers of cattle resulting in adverse financial implications (OIE, 2009). The test and slaughter policy has five impossible essential elements that are essential for its success in eradication programs which are strict interpretation of test results, uniformity of cattle production systems, availability of funds for compensation, sufficient government resources for eradication campaigns and an absence of BTB wildlife reservoirs (Smith et al., 2011). In areas or regions with the presence of risk factors for BTB, testing is done periodically to monitor and eliminate disease. The main obstacle to control and eradication efforts that has been encountered worldwide has been control of the disease in wildlife (Friyantha, 2008). Consistent with *M. bovis* control and eradication in Africa, wildlife poses an obstacle to complete eradication with so many wildlife species being implicated as maintenance hosts. In eradication programmes testing and slaughter remains the mainstay for success but this is hardly being practiced in most of Africa mostly due to lack of funding (Machado et al., 2018). South Africa follows this policy under its Tuberculosis Eradication

Scheme (South African Department of Agriculture Fisheries and Forestry) with a decreasing level of success particularly in livestock/wildlife interface areas. Egypt follows the policy without significant reduction in disease prevalence (Soliman et al., 2004).

## **2.6 Bovine tuberculosis in different parts of the world**

BTB occurs worldwide, by June 2018, 56% of countries and territories reporting to the OIE reported absence of the disease (OIE, 2018). In Africa, only Kenya has never reported it out of the whole sub Saharan Africa. Of all the continents of the world, Australia is the only one free from BTB (Rutten and Michel, 2014). Countries within the EU that are officially free from BTB following successful eradication programmes include Denmark which became free in 1980, Netherlands, Finland and Sweden in 1995, Germany and Luxembourg in 1997, Austria and Italy in 1999, France in 2001, Belgium in 2003 and the Czech Republic in 2004 (Gordejo and Vermeersch, 2006). Within Europe, the United Kingdom and Ireland still have a persistent BTB challenge within their production herds with the UK with an incidence of 4.5% by end of 2013 (Rutten and Michel, 2014).

## **2.7 *M. bovis* in Africa**

BTB is very widespread in Africa, but some countries fail or are erratic in reporting the prevalence and incidence of the disease (Ayele et al., 2004), therefore true epidemiological data are lacking or outdated (Machado et al., 2018). By 1992, 33 countries of the 44 countries in Africa reported BTB to the OIE with only Zimbabwe, Kenya, Namibia and Seychelles reporting freedom (Cosivi et al., 1995). In Africa BTB remains a health threat to both animals and humans as 85% of cattle and 82% of people live in areas where disease is prevalent and most of these countries lack sufficient resources to combat it both in humans and animals (Ayele et al., 2004). The situation is made worse for countries where the disease has been eliminated as domestic animals can still be infected with *Mycobacterium bovis* from feral and wild animals which are maintenance hosts of the disease (Ayele et al., 2004). BTB has been shown to be present in Southern Africa in free range wildlife in the African buffalo, the banded mongoose, greater kudu, small antelopes, *Kafue lechwe* antelopes in Zambia, of these, reservoir hosts have been found to be the buffaloes, greater kudu, the *lechwes* and possibly the common warthog (De Garine-Wichatitsky et al., 2013).

The impact which wildlife has on occurrence of BTB is high but cannot be quantified, because according to the OIE about 61% of African nations do not have any data on wildlife BTB (De Garine-Wichatitsky et al., 2013) . BTB has been found to be prevalent in cattle

producing areas in Ethiopia with increased prevalence where there is intensive and semi-intensive production, but unfortunately there are no effective control efforts being implemented in the country (Sibhat et al., 2017). Contrary to this, Durnez and co-workers found an increased prevalence of BTB in extensive production systems where there is free animal movement (Durnez et al., 2009). This was because there was an increased possibility of interaction with wildlife and other cattle as the cattle moved in search of grazing (Durnez et al., 2009). Recently there has been an increase in dairy farming in semi-urban areas in most developing countries which has had a negative impact on the epidemiology of disease leading to increased incidence of disease (Vordermeier et al., 2012). All these continue to hinder advances towards the control and eventual eradication of disease in Africa, where the majority of the population depend on their livestock particularly cattle for their livelihoods, ultimately with a negative impact on their health and finances.

It was also found that this prevalence is proportional to cattle age, decreasing animal body condition score, herd size as well as contact with wildlife (Dejene et al., 2016). In Tanzania a BTB prevalence range of 0.2% to 13.3% exists countrywide, but there are no effective policies to control the disease (Mugambi et al., 2016), whilst Mozambique has an estimated overall prevalence of 13.6% (Machado et al., 2018). Nigeria reports a current endemic BTB status with strong recommendations being made to establish efficient monitoring and surveillance control policies (Jajere et al., 2018).

## **2.8 *Mycobacterium bovis* in Namibia**

Namibia declared freedom from BTB with the last case having been recorded in 1995 in cattle, the disease was notifiable as far back as 1956 (Chitate et al., 2019). Since then, surveillance has been the only method used in Namibia to monitor and maintain the freedom status which is reported to the OIE. According to OIE standards, a country can have very low incidence of isolated cases at acceptable levels, a prevalence of 0.2% in herds and animal population prevalence of <0.1% and still be deemed disease free, therefore maintaining its freedom status (Sergeant et al., 2017).

Documented active and passive surveillance programmes started as far back as 1910 in Namibia. The main methods were divided into ante and postmortem surveillance systems, this era also saw the culling and elimination of most buffalo herds from Namibian soil as they were seen as a threat to animal health (Schneider, 1995). Ante-mortem surveillance was done by way of testing cattle using the single and comparative intradermal tuberculin tests. Postmortem surveillance involved examination for BTB lesions in lungs and lymph nodes of

the carcass at meat inspection, carcasses found positive for lesions were condemned, herd of origin traced and tested, positive reactors were slaughtered, and confirmation was done by isolation and culture of *M. bovis*.

Currently surveillance is at abattoirs whereby an accredited veterinary official performs an ante-mortem examination on cattle, ensuring that an animal is healthy with no visible clinical signs or suspicion of an infectious disease. At post mortem, according to the Namibian Directorate of Veterinary Services meat inspection guidelines, inspection involves visual appraisal, palpation and the making of prescribed cuts on the offals and carcass with visual detailed examination of lymph nodes including making of single or multiple incisions. As prescribed by OIE, BTB diagnosis is made at postmortem by identifying cattle with tubercles and swollen associated lymph nodes in abattoirs (Commission and Committee, 2008). In the event of only organ involvement the organ is rejected, but if lesions are widely distributed throughout the carcass with lymph node involvement, the whole carcass is condemned and the herd of origin is traced. Samples are collected and sent to the Central Veterinary Laboratory in Windhoek for confirmation by bacterial isolation and culture and subsequent disposal of carcass is done. If in uncertainty, additional multiple incisions are made and the carcass detained for further investigation. Information from all abattoirs in all regions is collected and compiled yearly to be published in an annual report by the Directorate of Veterinary Services. Whilst some schools of thought have argued that traditional meat inspection methods for detection of *M. bovis* have a questionable sensitivity, it has been found that visual inspection of carcasses and incisions on the lungs and lymph nodes have a 95% success in detection of macroscopic tuberculous lesions caused by the bacteria and therefore is highly reliable (Neill et al., 1994).

## **2.9 Beef trade in Namibia**

Namibia has been exporting beef to the EU since 1975 through the 'Lomé Agreement' which granted beef from Namibia duty free and a 90% reduction in tariff access to the EU markets, followed by the 'Cotonou Agreement' of 2000 (Strydom and Museler, 1996; Chiriboga et al., 2008). This has been influenced by freedom from notifiable diseases from the export herd. Namibia was the second country in Africa recognized by the OIE to have an FMD (Foot and Mouth Disease) free zone from which the export herd to the EU is derived (Strydom and Museler, 1996). The export herd is derived from regions south of the Veterinary cordon fence (Figure 2.1) which runs from the west to the east of the country. This cordon functions to control movement of animals across this boundary particularly from the north and north-west. Intense veterinary surveillance measures and regular FMD

vaccinations are done in the area north of the cordon fence whereas less intense surveillance is done in the south but with stringent animal movement control measures. North of the veterinary cordon fence is the FMD infected zone which is in the Zambezi region (formerly known as Caprivi strip) which encompasses wildlife protection areas, the buffer zone which borders the wildlife areas as well as the rest of the northern area where vaccination in cattle is done bi-annually, a surveillance zone immediately south of the veterinary cordon fence and the rest being the disease free zone.

The beef export regions are 5 in total namely Otjozondjupa, Omaheke, Khomas, a part of Oshikoto and Kunene and all of these are located south of the cordon fence as shown in Figure 2.1. Farming types in existence in these regions include commercial and communal with the type of cattle production being semi intensive and subsistence with the majority of farmers depending on cattle for their livelihoods. An estimate total of about 2.4 million cattle are produced in Namibia, of these, 1.5 million are produced in communal areas and 0.85 million are produced in commercial areas with 1.6 million being produced in areas south of the veterinary cordon fence (Meat Board of Namibia, 2009). Cattle produced south of the veterinary cordon fence can be exported directly overseas whereas farmers north of the fence do not enjoy this privilege. Moreover, their cattle should be quarantined for 21 days before slaughter and undergo mandatory chilling after slaughter for another 21 days before being allowed to be exported to neighbouring South Africa. As of 2011, of the cattle that were produced south of the veterinary cordon fence, 35% of income was derived from beef export with about 200 000 cattle slaughtered at export abattoirs, 17% from slaughtering for local consumption, 41% from exporting live cattle to South Africa and 7% from exporting to the north of the veterinary cordon fence (Namibian Livestock sector strategy final report, 2011).

There are two export abattoirs in Namibia certified by the European Union which are Witvlei Meats and Meatco, with the largest exporter to the EU being Meatco (Meat Board of Namibia) both located south of the veterinary cordon fence. These abattoirs are ISO and HACCP certified servicing all the export regions south of the veterinary cordon fence.

## **2.10 Game farming in Namibia**

In recent years there has been a shift in interests with close to a quarter of Namibian farmland being directed and converted into commercial game farming areas (Magwedere et al., 2012), whilst other farmers practice both livestock and game farming. Land use for wildlife based activities is over 287 000km<sup>2</sup> in Namibia and it has been found that private

farms have 21-33 times more wildlife as compared to committed wildlife protected areas (Lindsey et al., 2013).

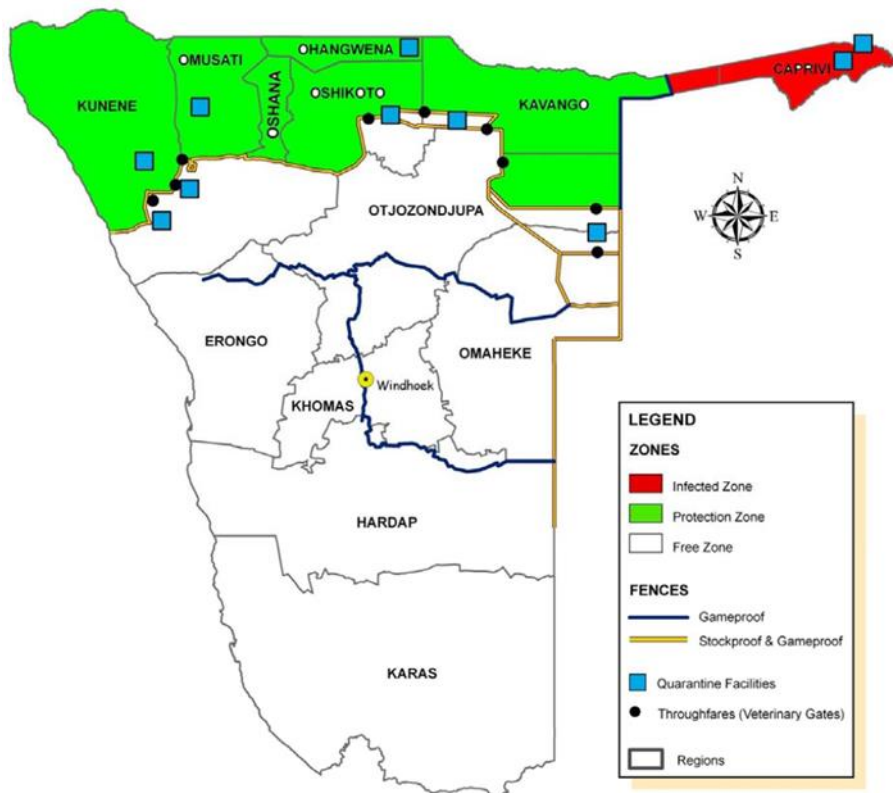


Figure 2.1. FMD zones in Namibia (adapted from Directorate of Veterinary Services: In FMD Disease Free Zones and Fences; Namibia)

This could be attributed to a decline in income from livestock production, limited farming incentives, an increase in hunting interests and increased ecotourism (Saltz et al., 2004). This has also probably been necessitated by the growing demand for venison both locally and externally, it was recorded that between 16 000 and 26 000 tons of game meat is produced in Namibia to serve national and international markets respectively (Lindsey, 2011). In some areas, ruminant wildlife species are reared in close proximity to livestock which potentially results in a spill-over of infections in either direction (Magwedere et al, 2012). As seen on the map of regions in Namibia in Figure 2.1, the presence of game proof fences in the export zone, south of the veterinary cordon fence cannot totally prevent some animals from damaging this barrier allowing free interaction of cattle and wildlife at such interfaces. The veterinary cordon fence is not completely animal proof either as big animals such as elephants can trample down these fences and some predators such as cheetahs

can traverse across creating temporary interaction of nearby cattle and wildlife before repairs can be done. BTB in Namibian wildlife has not been officially investigated, and these incidences may leave room for potential transfer of *M. bovis* to the cattle export herds. Surveillance for *M. bovis* in wildlife has proven to be a cumbersome task (Maas et al., 2013) because of the need to capture animals and possibly putting them in temporary confinement, thus most wildlife species are diagnosed at post mortem after being trophy hunted, culled or killed in road accidents. Furthermore the diagnostic tests available are very few and have not been validated for all animal species, making surveillance in wildlife difficult (Rutten and Michel, 2014). Therefore with BTB's insidious nature the infected wild animals even if few can propagate infection to vast susceptible herds only to be diagnosed at post mortem meat inspection.

### **2.11 Risk factors for Bovine Tuberculosis**

There is a diverse array of risk factors of exposure to BTB in cattle which have been identified worldwide, most of them being interconnected. A history of BTB prevalence in a country, area or region is a significant risk factor of exposure to take into account at all times (Humblet et al., 2009). The survival of the pathogen is dependent on the weather and favourable environmental conditions in the particular area which are risk factors to be considered also taking into consideration the geographical location of the area (Jackson et al., 1995). Depending on the geographical location and feed storage practices, feeding of silage and hay to cattle exposes them to risk of BTB since badgers, well known reservoirs of *M. bovis* are known to contaminate the feed (Garnett et al., 2002). The absence of disease monitoring strategies such as abattoir surveillance, routine quarantine and testing of cattle for BTB pose risk of exposure to disease both in cattle and humans. Active eradication programmes eliminate cattle with BTB from the population hence lack of these programmes increases risk of exposure, particularly in countries with BTB. The BTB status regionally or across national boundaries must be taken into consideration as risk factors, particularly if the disease is known to be endemic. Other livestock such as pigs and sheep, although they can be infected and transmit BTB to cattle, appear to pose a low risk, as BTB has been found to be self-limiting in pigs, very rare in sheep and a variant, different from *M. bovis* has been found to infect goats (Aranaz et al., 1999).

At herd level, cattle density which is directly proportional to herd size is a risk factor which can be also be facilitated by some farming practices such as communal herding, transhumance and agro-pastoralism (Dejene et al., 2016). The resultant close interaction between cattle herds of unknown BTB status directly or indirectly risks exposure to disease

as opposed to free ranging individual cattle herds (Menziés and Neill, 2000). The cattle production systems practiced contribute to exposure to disease. Intensive production systems found in the developed countries such as Ireland whereby a large number of cattle are raised in closed confined spaces pose risk of rapid aerosol transmission of *M. bovis* (Woodroffe et al., 2005). Production systems whereby cattle are artificially inseminated or serviced by semen from animals of unknown BTB status can result in transmission of infection. Beef herds were found to be at lower risk of exposure to BTB as compared to dairy herds since they have a short lifespan as they are slaughtered for meat as soon as they reach target weight, unlike dairy cattle which must be productive for a longer time (Humblet et al., 2009). Cattle movement is another risk factor for exposure, this can be trans-border or local. Risk has been found to be greater if movement is from an area or region where BTB is prevalent (Gopal et al., 2006). New cattle introductions into herds without testing pose risk to the rest of the cattle within the herd.

At individual animal level, breed, age, parity and a poor body condition score are risk factors for BTB, particularly in areas where the disease is endemic. Indigenous breeds such as the Zebu cattle have been found to be genetically more resistant to BTB infection, whereas exotic breeds such as the Holstein Friesian were found to be more susceptible to *M. bovis* (Omer et al., 2001). Increasing age, which is directly proportional to parity is a risk factor for exposure to BTB since the likelihood of having been exposed to the pathogen increases with age or parity. Dairy cows of mean age 4.5 years were found to have a higher prevalence of BTB due to prolonged high productivity and husbandry type (Cousins, 2001). Wildlife contact, direct or indirect, which can be through sharing of grazing and water sources serves as a significant risk factor worldwide (Sergeant et al., 2017). Another risk of exposure to BTB, though uncommon is the close interaction of people infected with tuberculosis caused by *M. bovis* with cattle (Humblet et al., 2009). Infected people have been found to be a source of re-infection in some nations where BTB has been eradicated (Messinger and Schmeider, 1989; Anon, 1994). Food consumption behaviour such as raw milk consumption serves as a risk factor of exposure to BTB in humans.

## **2.12 Questionnaire design**

A questionnaire is a tool developed from a set of questions used to obtain clinically and statistically useful information from an individual. The questions are assembled into a logical, clear instrument that flows naturally and will keep the respondent sufficiently interested to continue cooperation (Abawi et al., 2013). Types of questionnaires are computer questionnaires, telephonic questionnaires or surveys, in house surveys, hand delivered and

mail questionnaires. Questionnaires can have four different types of questions; open questions whereby the respondents express their views in a free flow manner; multiple choice questions; dichotomous questions with two predetermined responses, and lastly, scaling questions whereby the response is put on scale accordingly.

## **3. CHAPTER 3**

### **METHODOLOGY**

#### **3.1 Research design and approach**

The research design was in the form of a cross sectional questionnaire survey of a representative sample of farmers within the 10 cattle raising regions in Namibia. The type of data of interest collected was qualitative and was used to describe and interpret exposure factors to BTB. As for this study design, questionnaires with multiple choice questions were developed and these were either hand delivered and or sent through electronic mail. Stages involved in designing the questionnaire included three distinct phases; initial questionnaire planning, development of specific questions and the final construction of the data collection instrument as a whole. Important aspects of the questionnaire were that each question provided a valid and reliable measure and the questions clearly communicated the research intention to the respondent. It was imperative to construct a good questionnaire, because whilst this is difficult, on the other hand, bad questionnaires are difficult to analyze (Boynton and Greenhalgh, 2004).

#### **3.2 Research population**

The target population for the questionnaires were farmers in 10 cattle producing regions of Namibia namely Kunene, Oshana, Omusati, Ohangwena, Oshikoto, Kavango, Zambezi, Khomas, Omaheke and Otjozondjupa. The regions are shown in the map in figure 3.1 below. There were about 2.77 million cattle in Namibia as of 2015, cattle farmers being the target population for the research from which the sample was derived (Agriforum, 2018).



Formula: Sample size  $N = \frac{(\text{confidence level at } 95\%)^2 \times \text{variance of population}(pq)}{\text{Allowable error } (\pm 5.5\%)}$

### **3.5 Data Collection Techniques and Tools used**

A questionnaire with 23 questions in three sections (Appendix 7) was designed, the front page showing the learning institutions at the top followed by the title which was 'Questionnaire survey' followed by a unique questionnaire number. The instructions details to guide the participant followed thereafter. The questionnaire had questions, some in two or more parts, which were numbered alphabetically in sequence. The questions had multiple responses to choose from which were assigned Roman numerals accordingly. Three of the questions were open ended, therefore respondents could fill in their own words.

The first section (Section 1) was on farm demographics and this had 5 questions asking the farming region, distance from the nearest border, type of farming, cattle herd size and other livestock and other specific animals owned by farmers, apart from cattle. The second section (Section 2) was on farming practices comprising eight questions. The first five questioned the presence or absence of fencing around individual farms, interaction between different cattle herds, sharing of grazing and water sources, names of wildlife seen and frequency of sighting of wildlife by farmers and distance cattle moved searching for grazing. The last three were asking farmers about the number of cattle sold, bought and received as gifts in the previous year, the markets the cattle were sold to and whether cattle were quarantined after purchase. The last and third section (Section 3) was on disease awareness consisting of ten questions. The first three asked if farmers' cattle had been tested for BTB and if they were familiar with the disease including its zoonotic potential. Thereafter, six questions included farmer knowledge on reasons for carcass or organ condemnation in the past, age groups of people consuming milk and milk drinking practices, frequency of slaughter of cattle, sheep and goats for home consumption, whether the meat was subjected to meat inspection and lastly, farmer actions when faced with an abnormality in a carcass. The last two questions were to ascertain the current situation and history of human tuberculosis cases within farmers' households and workers. At the end, the questionnaire thanked each participant. The same questionnaire was programmed electronically whereby check boxes were inserted for participants to select their responses.

A farmer consent form (Appendix 6) and letter of invitation (Appendix 5) were also designed. The farmer consent form required the signature of the farmer if they voluntarily agreed to participate in the survey. The letter of invitation, with the official University of Pretoria logo, introduced the principle investigator and explained in brief the disease and purpose of the study.

### **3.6 Justification of Data collection methods**

Prior to designing the questionnaire for the research, information required to achieve the set goals was thoroughly researched through reviewing of data. These areas of interests are demographics, livestock and farming aspects and all potential risk factors for BTB as published in literature. The target population for the questionnaire survey was identified, that is, the farmers who produce cattle. The method that would be most successful to reach the farmers was identified as delivery by hand, which ensures better interaction, by electronic mail would allow the questionnaire to reach most respondents who may not be able to be seen in person. The question content of the questionnaire was determined to use only relevant information so as not to overload the questions. The question wording was determined for ease of question answering and getting the desired relevant responses and not to offend or discourage participants. Questions were ordered and put in a format such that each participant received the same stimuli. The length was also made short and precise for efficiency thus encouraging participation. The questionnaire was translated into the various predominant local languages. It was then tested before being sent out to determine if it could serve its purpose throughout a randomly chosen group of people. Afterwards the final questionnaire survey form was made. Translation was justified as there are farmers who do not understand the English language therefore it gave them freedom to answer the questionnaire in a language they are comfortable with.

### **3.7 Data Collection Procedures**

The questionnaire reached the respondents by electronic mail and was distributed by hand by the principal researcher in each region of interest. The Emerging Commercial farmers Union of Namibia provided contact details of cattle producers in their database, which enabled the questionnaire to be sent through electronic mail. The questionnaires distributed by hand by the principal investigator were responded to immediately and collected soon after the end of the session. Translated questionnaires were made available for farmers who did not understand the English language. For each respondent whose questionnaire was hand distributed, the investigator gave a brief background of the

purpose of the questionnaire survey, expectations and the voluntary nature of the exercise. The informed consent form together with the questionnaire and a pen were then handed and respondents invited to respond to the questionnaire and to ask for clarification or assistance at any stage if required. On completion, the consent form and questionnaire were collected and respondents thanked and investigator departed.

Electronic questionnaires and consent form were sent to the recipient, these had a check box which allowed them to agree or disagree with the consent and answering the questionnaire by ticking the appropriate response. Afterwards the documents were sent back to the principal researcher's electronic mail address where they could be downloaded and filed. After the research period, the questionnaires were collected and evaluated to meet the objective of the study.

### **3.8 Measures to Ensured Validity**

To ensure that the questionnaire survey was going to be valid, a pilot project was carried out. This model has been shown as a fast, effective way of reaching a large population and is explicit in nature as it identifies animals with the most risks and thus highly likely to have disease. This has been shown to be explicit in releasing information pointing towards disease occurrence (WHO, 2011). Also, according to Sergeant and co-workers (2017), this was a reasonable assumption in investigating disease as opposed to testing of all animals in the entire population for BTB with a perfect test (Sergeant et al., 2017).

#### **3.8.1 Piloting**

A pilot project was held before commencement of survey to ascertain suitability and reliability of the questionnaire survey. Ten farmers, a mixture of communal and commercial farmers of different dialects (English, Afrikaans, Oshiwambo, Herero, Silozi, Damara and Mbukushu) were invited for the piloting at the same time. The principal investigator gave them a brief background of the purpose of the survey and informed them about the expectations and the right to freedom of consent. Consent forms and the questionnaires were distributed and then they were asked to answer the questionnaires in the selected languages, thereafter, upon completion, English questionnaires were handed to the respondents so that they could cross check if the interpretation of the questions were the same. Consent forms and questionnaires were collected upon completion for perusal of every questionnaire to determine the level of success of the survey, a discussion was held to ascertain any challenges that could be anticipated and to identify areas for rectification

and improvement. Concerns with regard to the questionnaires that could have been raised by respondents were highlighted and the necessary modifications done.

### **3.9 Measures to Ensured Reliability**

The questionnaire was structured into sections whereby each section had questions which allowed cross checking to see the truthfulness in the answer. Firstly, the farming region could cross check with the anticipated closest border to the farm. The type of farming could cross check with the number of cattle or herd size, the presence or absence of a fence around the individual farm and the interaction of that herd with neighbouring cattle. The type of farming also cross checked with the amount of cattle sold each year and the market to which the cattle are sold to and whether or not they passed through abattoirs. For certain questions, if the respondent's answer was not given, there was an allowance for them to write down their response in spaces provided, this ensured that respondents were not cornered into making incorrect responses.

### 3.10 Data analysis

Questionnaire survey data were analyzed through direct compilation and comparison of data using qualitative descriptive statistical methods. Results of compiled data were presented by way of bar graphs and pie charts. A comparison of the distribution of exposure factors between communal and commercial farm areas was presented by way of bar graphs. A risk factor was classified as negligible if it was so rare that it did not merit any consideration and very low, low, medium, high and very high were further classifications used as summarized and interpreted in table 3.1 below.

Table 3.1. Risk level classification (adapted from EFSA, 2006)

Risk Category	Interpretation
Negligible	So rare that it does not merit to be considered
Very low	Very rare but cannot be excluded
Low	Rare but does occur
Medium	Occurs regularly
High	Occurs very often
Very high	Events occur almost certainly

### 3.11 Ethical considerations

#### 3.11.1 Informed consent

Informed consent to carry out study was obtained from the Ministry of Health and Social Services of Namibia Research Division (Appendix 1), Animal Ethics Committee (Appendix 2) and Research Ethics Committee (Appendix 3 and 4) at the University of Pretoria, the certificates of approval are shown in Appendices. Responding to the questionnaire survey was entirely voluntary. There was no conflict of interest associated with the research project.

#### 3.11.2 Guiding principles of ethics observed

#### 3.11.3 Preservation of life

Life was preserved at all times, the questionnaire survey itself did not have aspects that posed danger to life, however, instances whereby there could be conflicts between people could arise and all efforts were made for the atmosphere to be peaceful and tranquil at all

times by being polite and respectful of people across all groups.

#### **3.11.4 Principle of beneficence**

The questionnaire survey aimed to gather information about BTB, and at the same time raised farmer awareness of the disease and exposure factors, which could help them in identifying sick animals promptly for veterinary assistance thereby reducing economic losses.

#### **3.11.5 Principle of confidentiality**

The questionnaire did not ask for any personal details of any kind that could be used later in breach of confidentiality. The consent form allowed for a signature only, however, if a respondent felt that they could not sign they could leave the signature out and respond to the questionnaire. The participants were well informed that the data collected were only for academic purposes as indicated in the consent form, no names or personal details were to be published and questionnaire material was to be destroyed permanently after data compilation.

#### **3.11.6 Principle of veracity**

The survey avoided deceptive practices at all costs, participation was entirely voluntary thus leaving no chance of coercion or intimidation of participants. There was freedom of expression with a participant free to stop completing the questionnaire at any stage or not respond to some questions.

#### **3.11.7 Principle of non-maleficence**

Minimizing the risk of harm was observed in all instances, there was no anticipated physical harm on the respondents; however, emotional harm could arise if respondent was not comfortable with the questionnaire. The survey allowed for voluntary consent to participation and participants stopped at any stage if they felt they were not able to carry on regardless of the reason. This was made clear within the consent form and was explained to participants before the questionnaire was distributed.

#### **3.11.8 Principle of fidelity**

All agreements were adhered to and these were reinforced with the consent form, which was signed therefore available as legal liability.

#### **3.11.9 Principle of upholding justice**

There were no variations in administration of the questionnaires neither were special

favours granted to certain people. All participants received the same kind of invitation, background summary and stimuli.

#### **3.11.10 Principle of respecting autonomy**

There was respect of people's individual reactions and decisions, participants were given a chance to make their own decisions and agree or disagree to respond in the first place as guided by the consent form. They were aware that they could withdraw from the study at any point without being questioned or made to feel guilty. Disadvantaged participants who did not understand English were provided with a translated questionnaire which they could understand.

## 4. CHAPTER FOUR

### RESULTS

#### 4.12 Farm demographics

A total of 254 farmers from the 10 cattle raising regions of Namibia participated in the questionnaire survey. As shown in table 4.1 below, with the exception of the Omaheke and Khomas regions, which together contributed to less than 10% of the participants, the other eight regions were almost equally represented (10.2% to 11.8%) in the survey.

Table 4.1. Distribution of farmer participants across regions

Region	Number of farmers	Proportion of farmers (%)	95% Confidence interval
Oshikoto	30	11.8	7.87-15.73
Kunene	30	11.8	7.87-15.73
Oshana	30	11.8	7.87-15.73
Otjozondjupa	30	11.8	7.87-15.73
Zambezi	30	11.8	7.87-15.73
Kavango	28	11.0	7.32-14.68
Omusati	27	10.6	7.92-13.28
Ohangwena	26	10.2	7.84-12.56
Omaheke	13	5.1	2.42-7.78
Khomas	10	3.9	1.54-6.26

#### 4.12.1 Border proximity

Namibia has 4 neighboring countries sharing its borders, namely Angola, Zambia, Botswana and South Africa. Farmers who indicated proximity to the Botswana border were 35 (14.3%), 130 (53.3%) were close to the Angolan border and 22 (9%) to the Zambian border. However, 57 (23.3%) indicated absence of proximity to any border within 200 km from their farms. Most of the farmers, 74 (38.5%) had farms located at least 100 km from the border, whilst the minority of farmers who contributed less than 10% were at most 10 km from the nearest border, the rest of the farms (46,5%) were at least 10 km away from the border as shown below in figure 4.1.

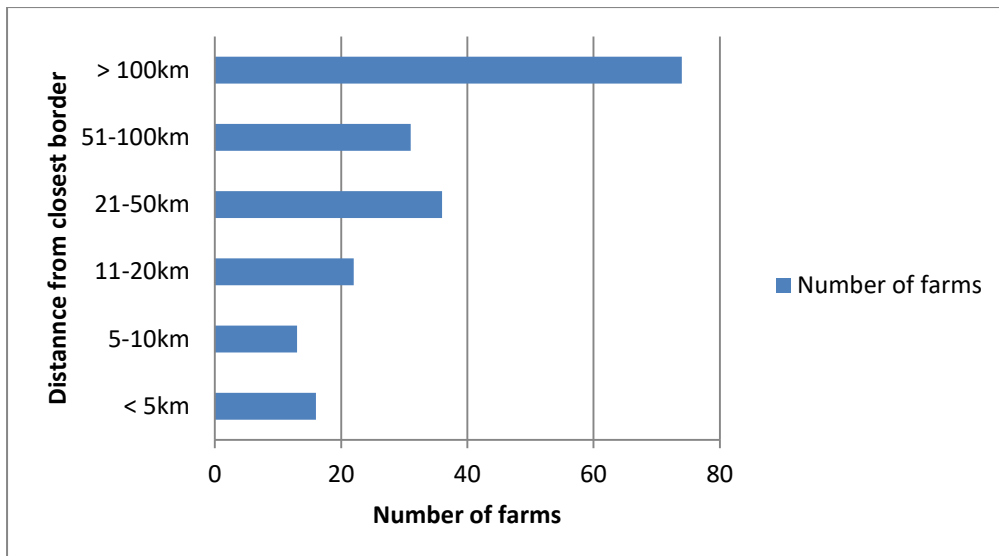


Figure 4.1. Distance to closest international border

#### 4.12.2 Type of livestock farming

Of the 254 farmers who responded to the questionnaire, 72 (28.3%) indicated that they were commercial farmers and 178 (70%) indicated that they were communal farmers, however, 4 (1.7%) indicated both options, therefore indicating they practice both types of farming as they owned both commercial and communal farms, as shown in figure 4.2 below.

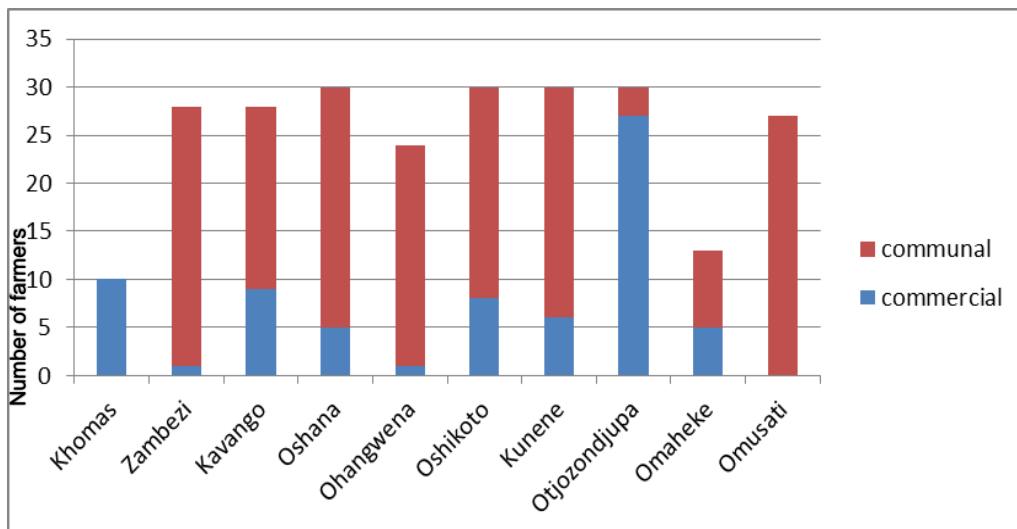


Figure 4.2. Types of cattle farming across regions

### 4.12.3 Cattle herd size

The vast majority of communal farmers (52.7%) had a herd size of between 5 to 20 whereas most commercial farmers (95.8%) had herd sizes greater than 50 cattle as illustrated in detail in figure 4.3.

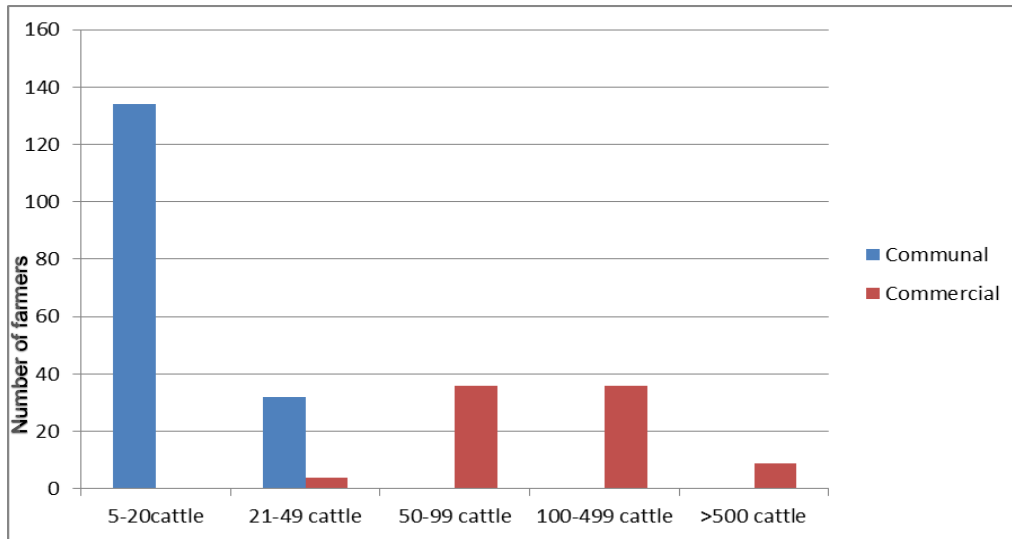


Figure 4.3. Sizes of cattle herds in commercial and communal farming systems

### 4.12.4 Livestock owned apart from cattle

Most of the farmers, 172 (69.9%) in total had other livestock apart from cattle, while 74 (30.1%) had no other livestock. Apart from cattle, goats formed the majority of other livestock owned 157 (76.9%) across commercial and communal farmers, sheep were owned by 36 (17.6%) commercial farmers, free ranging pigs were owned by 10 (4.9%) communal farmers and only 1 farmer owned other unnamed livestock.

#### 4.12.5 Wildlife ownership and sighting

Table 4.2. Wildlife ownership and sighting across farming regions

Farming region	Farmers owning wildlife	95% Confidence interval	Farmers sighting wildlife	95% Confidence interval
Khomas	5 (14.3%)	10.04-18.56	10 (7.9%)	4.62-11.18
Oshikoto	3 (8.5%)	5.11-11.89	18 (14.3%)	10.04-18.56
Otjozondjupa	8 (22%)	16.96-27.04	24 (19%)	14.23-23.77
Kunene	2 (5.7%)	2.88-8.52	17 (13.5%)	9.34-17.66
Omaheke	1 (2.8%)	0.79-4.81	10 (7.9%)	4.62-11.18
Ohangwena	0	0	5 (3.9%)	1.54-6.26
Zambezi	6 (17.1%)	12.52-21.68	16 (12.7%)	8.65-16.75
Kavango	7 (20%)	15.13-24.87	8 (6.3%)	3.34-9.26
Oshana	1 (2.8%)	0.79-4.81	13 (10.3%)	6.6-14
Omusati	0	0	4 (3.1%)	0.99-5.21

Of the farmers interviewed, 35 (13.8%) confirmed owning wildlife apart from cattle, of these, 19 (26.4%) were commercial farmers. The majority of the farmers owning wildlife were from Otjozondjupa (22.8%) and Kavango (20%) regions, whilst none of the farmers from Ohangwena and Omusati regions owned any wildlife, as shown in table 4.2. Apart from farmers owning wildlife, farmers who saw wildlife on and around their farms were 126 (49.6%). The lowest number of these farmers, less than 10, were from Kavango, Omusati and Ohangwena regions, whilst the rest of the regions had at least 10 farmers seeing wildlife as shown in detail in table 4.2 above.

#### 4.12.6 Wildlife and cattle interaction

The wild animals observed the most were warthogs, other animals seen were elephants, giraffes, springbok, jackals, foxes and hippopotamus as presented, in table 4.3. The farmers that responded positively to their cattle sharing grazing with wild animals were 73/254 (28.7%) whilst 85/254 (33.5%) of the farmers' cattle shared a water source with wild animals. The general frequency of seeing these wild animals was varied with most farmers, 32 (24.4%) seeing them daily, however, the frequencies of the rest of the time intervals were well within a close range of 16.8% to 19.8% as depicted in figure 4.4.

Table 4.3. Wildlife observed on farmland

Wild animals species	Number of farmers seeing wildlife	95% Confidence interval
warthogs	51 (40.5%)	34.53-46.47
kudus	48 (38%)	31.92-44.08
mongooses	8 (6.3%)	3.34-9.26
wildebeest	10 (7.9%)	4.62-11.18
meerkats	22 (17.5%)	12.88-22.12
others	10 (7.9%)	4.62-11.18

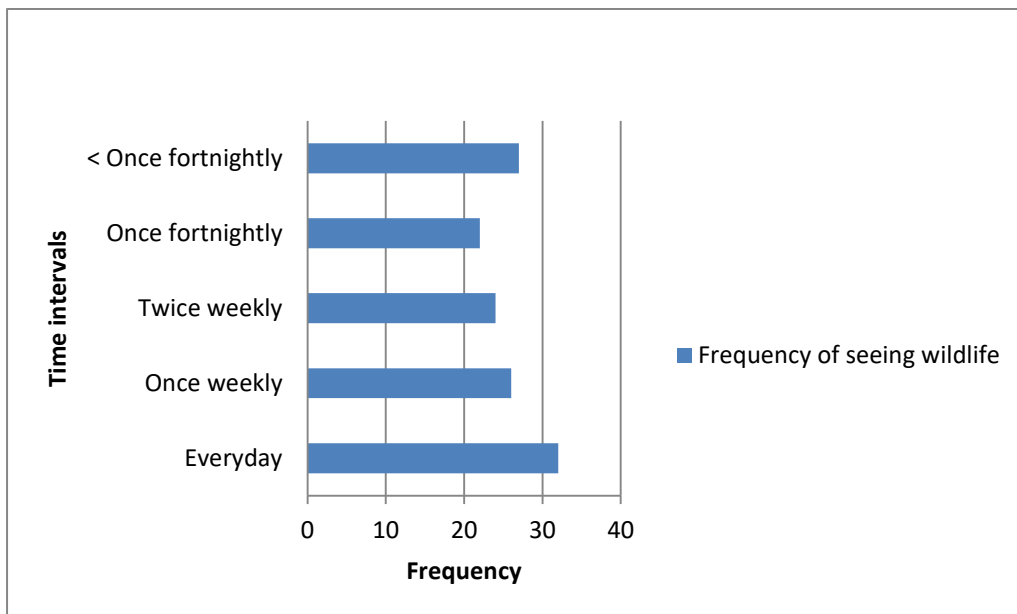


Figure 4.4. Frequency of observing wildlife

#### 4.13 Farming practices

The farmers who indicated that their farmland was completely fenced were 149 (58.7%) including all commercial farmers, 24 (11.8%) of the farmers had partial fencing and 75 (29.5%) had no fencing at all around their farmland. It was found that 99 (39%) farmers had their cattle grazing with other neighbouring cattle, which was attributed to communal herding practices, lack of complete fencing and no fencing. The majority of farmers, 86 (34.3%) in total had pumped water as their main water source, 60(23.9%) of the farmers' water sources were man made waterholes, 66 (26.3%) farmers used rivers and 39 (15.5%) had dams as water sources for their animals.

#### 4.13.1 Distance moved by cattle in search of grazing

There is little rainfall throughout the year in Namibia, which translates to poor pasture therefore in communal areas cattle have to move away from the homesteads in search of pasture. Similarly, extensively raised cattle in commercial farms move further into the farmlands to find better pasture. Most of the farmers (77.2%) had their cattle moving at most 10 km in search of grazing, while the lowest number of farmers (7.1%) responded that their cattle searched for grazing for more than 20km as shown in table 4.4 below.

Table 4.4. Farmers responses to distance moved by cattle in search of grazing

Distance moved (km) by cattle	Number of farmers	Percentage (%)	95% confidence interval
<5 km	111	43.7	37.66-49.74
5-10 km	85	33.5	27.76-39.24
10-20km	31	12.2	8.22-16.18
>20 km	18	7.1	3.98-10.22

#### 4.13.2 Cattle trade

The information on cattle that had been sold, bought and acquired as gifts within the previous year was obtained from the farmers. The highest number of farmer participants (59%) sold cattle in the range of 1 to 10 animals, only 11.8% of farmers sold a large number of cattle (more than 50 animals per farmer), a small number of farmers amounting to less than 5% sold animals within the range of 11 to 50 in number. With the exception of 30% of farmers, the majority being commercial farmers, a vast majority of farmers, almost 70% had not bought any cattle in the previous year. Most of the farmers (84.2%) never received cattle as gifts, of the farmers that received cattle as gifts within the previous year, the majority (72.7%) were communal farmers.

Of the cattle sold, the majority of the farmers (55.3%) had sold to the local market while 15.5% of farmers, who were all communal farmers, were uncertain as to which market had bought their animals, as shown in figure 4.5. The farmers who had sold cattle within the previous year included 83.3% of the commercial farmers who sold to Meatco, Hartlief, and the South African markets. None of the commercial farmers were uncertain about their cattle markets (figure 4.5).

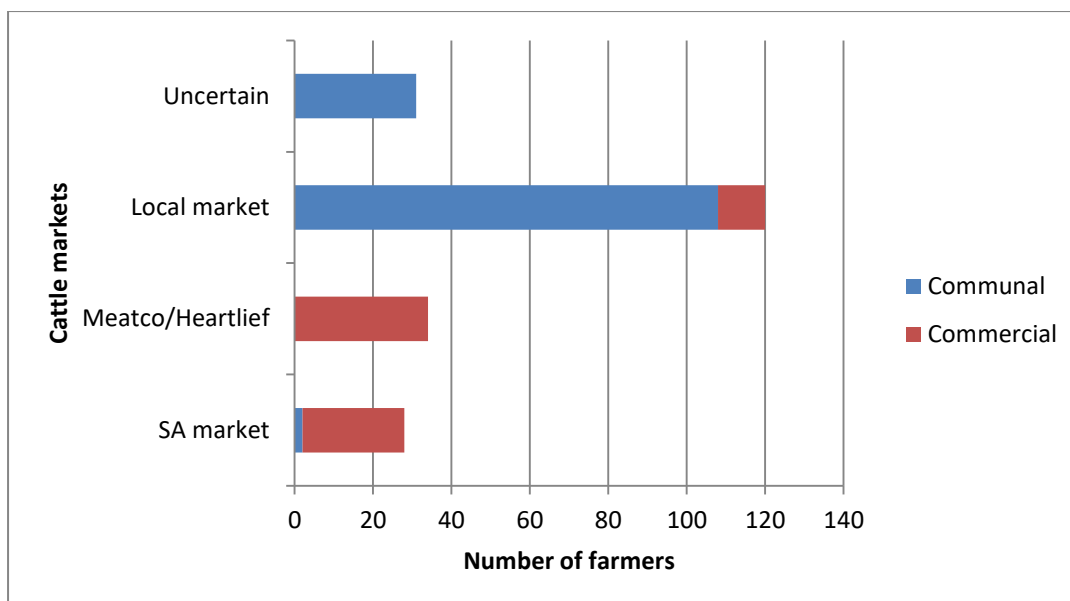


Figure 4.5: Target markets for cattle sold

#### 4.13.3 Disease awareness and prevention

The majority of the farmers, 136 (55.5%) had no knowledge of BTB whilst 109 (44.5%) farmers were familiar with the disease. On further investigation, 96 farmers (39.5%) knew that BTB could affect humans, 60 (24.7%) thought the disease could not affect humans and 87 (35.8%) farmers were uncertain about its zoonotic potential.

#### 4.13.4 Testing and quarantine of cattle

The majority of farmers (68.8%) indicated that their cattle had never been tested for BTB in the past, Oshana (7%) and Kunene (14%) regions had the least farmers whose cattle had been tested for BTB whilst the rest of the regions had at least 20% each. The regions where at least 70% of the farmers' cattle had been tested for BTB were Khomas and Kavango, as shown in detail in table 4.5. With the exception of all farmers in Khomas region and a small number of farmers in 7 other regions, the majority (77.7%) of farmers interviewed including all farmers in Oshikoto and Oshana regions had new cattle being immediately introduced into their herds without any quarantine as presented in table 4.5 below.

Table 4.5. Application of BTB testing and quarantine

Region	Number of farmers with cattle quarantined As fraction of total quarantined	95% CI	Number of farmers whose cattle were tested per time period					
			≤1 yr	≤2 yrs	≤3 yrs	>3 yrs	Total per region	95% Confidence interval
Khomas	10 (19%)	14.23-23.7	0	1	2	6	9(90%)	87.76-92.24
Omaheke	6 (11.3%)	7.45-15.1	0	0	1	4	5(38%)	36.31-39.69
Kunene	2 (3.8%)	1.47-6.13	0	0	4	0	4(13%)	11.49-14.51
Otjozondjupa	4 (7.5%)	4.3-10.7	2	1	1	4	8(23%)	20.87-25.13
Oshikoto	0	0	4	0	0	4	8(27%)	24.87-29.13
Ohangwena	5 (9.4%)	5.85-12.9	3	0	4	0	7(30%)	28.01-31.99
Zambezi	9 (17%)	12.43-21.6	4	1	0	1	6(20%)	18.15-21.85
Kavango	12 (22.6%)	17.51-25.7	11	3	2	4	20(71%)	67.72-74.28
Oshana	0	0	0	0	0	2	2(7%)	0
Omusati	5 (9.4%)	5.85-12.9	4	2	1	1	8(30%)	24.42-35.58

#### 4.13.5 Meat inspection history

Of the farmers who had sent their cattle to abattoirs for slaughter in the past, 40 (17.1%) confirmed that some of their cattle parts or organs had been condemned, 146 (62.7%) did not confirm in the affirmative while 47 (20.2%) were uncertain if their cattle were ever condemned at all. The majority of condemnations were of unknown causes 18 (30.5%) and lumpy skin disease 13 (22%), while other causes were abscesses 10 (16.9%), pneumonia 7 (11.8), bovine measles 5 (8.5%) and contagious abortion 2 (3.4%). Other specific causes of condemnation highlighted by 4 (6.9%) farmers were bruising, jaundice, cysts and abnormal lungs.

#### 4.14 Food consumption behaviour

Consumption of milk from cattle was found to be an uncommon practice as 166 (67.7%) farmers never or rarely consumed any milk at all. However, most of those consuming milk did so daily, whilst the frequencies of consumption twice weekly and once a week were almost equal as depicted in figure 4.6.

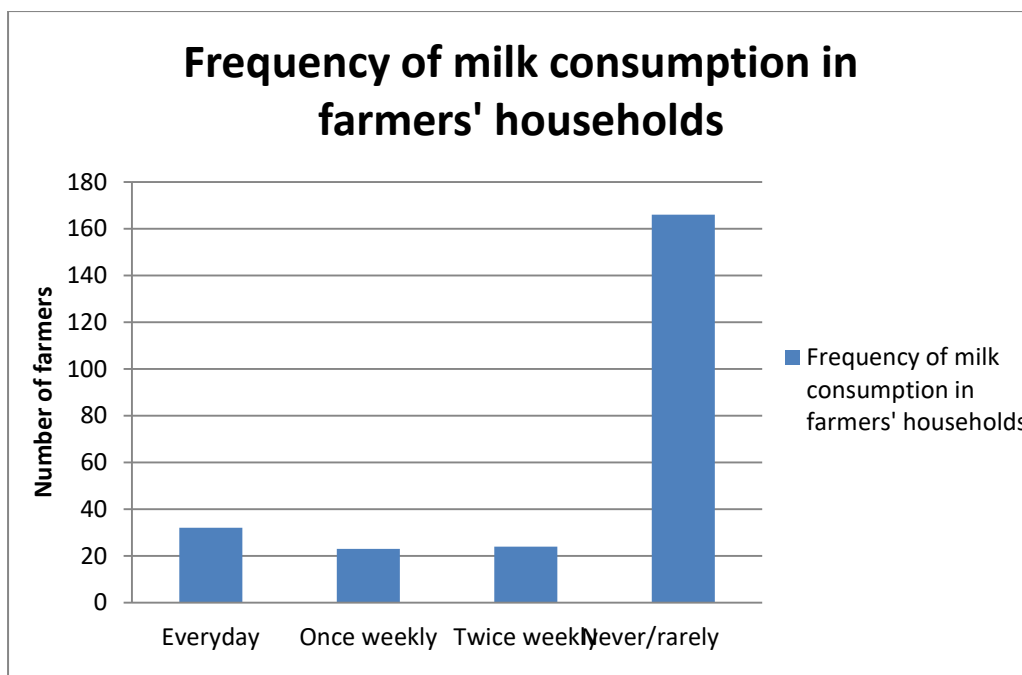


Figure 4.6. Frequency of milk consumption in farmers' households

The frequency of farmers indicating the various age groups consuming milk in their households was ascertained. The age group with the highest frequency (n=114) of milk consumption was children less than 12 years old, the elderly older than 65 years old were indicated to have the lowest consumption (n=40) and the frequency in the remaining age groups was almost twice that of the elderly, as depicted in table 4.6 below. Of the milk consumed, most of the farmers (49.8%) indicated that the milk was eaten soured, 31% said it was boiled first and 19.2% responded that milk was consumed raw.

Table 4.6. Milk consumption per age group

Age group	Frequency of farmers indicating milk consumption (n=x) in their households
<12 years	n=114
12-18 years	n=77
18-65 years	n=86
>65 years	n=40

#### 4.14.1 Livestock slaughter for home consumption

Livestock slaughter for home consumption was investigated among the farmers and the frequency of slaughter established. The frequency of home slaughter ranged from once a month, once in 3 months, once in 6 months, once or twice a year and never or rarely. The frequency of never or rarely slaughtering was high for cattle (n=91), sheep (n=18) and for goats slaughter once to twice a year (n=38) as seen in table 4.7. The frequency of slaughter was lowest for once a month in cattle (n=10), in goats (n=22) whilst it was lowest for sheep slaughtered once in 6 months as depicted in table 4.7.

Table 4.7. Frequency of slaughter of livestock for home consumption

Time frame	Frequency of slaughter of animals for home consumption (n=x)		
	Cattle	Goats	Sheep
Once a month	n=10	n=22	n=16
Once in 3 months	n=24	n=26	n=16
Once in 6 months	n=34	n=28	n=9
Once to twice a year	n=48	n=38	n=11
Never/rarely	n=91	n=30	n=18

Of all of the farmers slaughtering their animals for home consumption, only 24 (10%) had a veterinary official inspecting the meat, whilst the majority, 217 (90%), had no meat inspection done. In the event that farmers found an abnormality within a carcass intended for home consumption, the responses were very varied, with most of the farmers (30%) taking the abnormal part or organ to the nearest veterinarian and the lowest number of farmers (2.8%) confirming that they would ignore and prepare as normal. The rest of the farmers (68.7%) were those who confirmed to cutting the abnormal part and discarding in the veld, cutting and burning the abnormal part, cutting out the abnormal part and burying into the ground, cutting and feeding it to the dogs or simply burning the organ as depicted in figure 4.7 below.

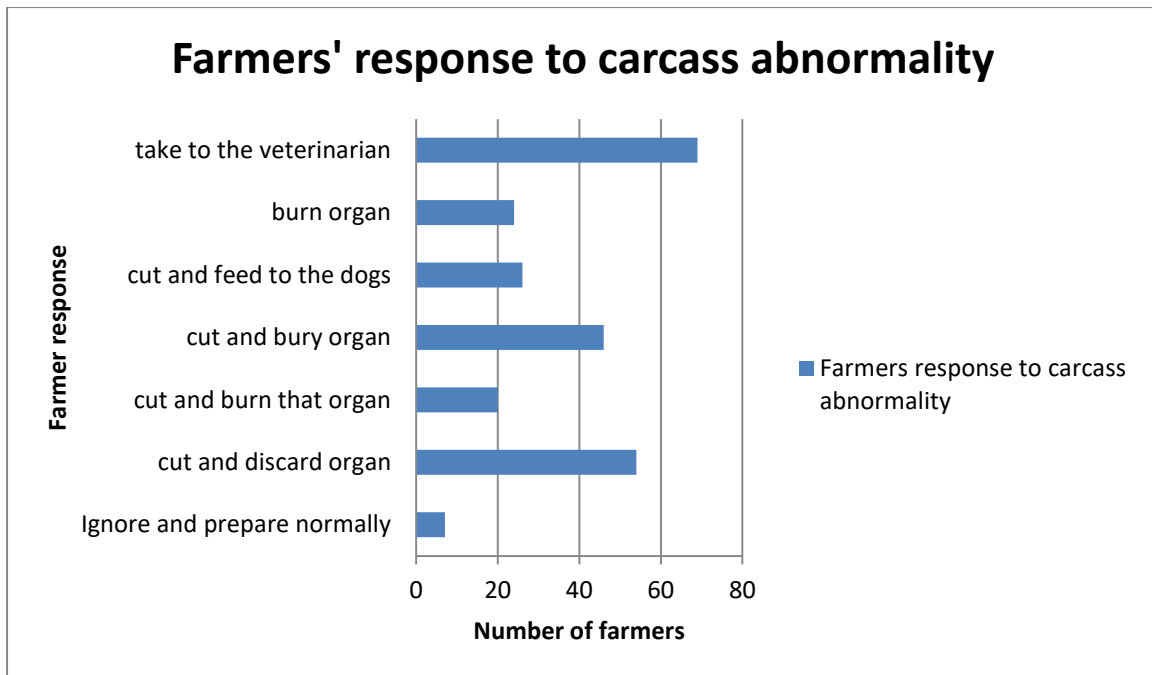


Figure 4.7. Farmers' response to abnormal organ/ carcass after slaughter

#### 4.15 History of human tuberculosis within farmers' households

The history of exposure to human tuberculosis within the farmers' households was investigated; 32(12.6%) farmers confirmed that they had a worker or household member who was currently being treated for the disease and 44 (11.73%) farmers confirmed that they had a worker or member of household with a history of human tuberculosis illness.

## **5. CHAPTER FIVE**

### **DISCUSSION**

#### **5.1 Introduction**

The study was the first to investigate risk factors of BTB in the cattle population in Namibia. The method used to investigate the risk factors was by way of a questionnaire survey to obtain data from participating farmers on farm demographics, farming practices and disease awareness with the objective of investigating and qualitatively describing the BTB risk factors across the farming types and their relative importance in Namibian cattle herds.

The project was performed in the 10 cattle farming regions of Namibia, namely Khomas, Otjozondjupa, Kunene, Oshikoto, Kavango, Omusati, Oshana, Zambezi, Ohangwena and Omaheke. Both commercial and communal farmers from different settings participated in the study.

A sample size of 300 farmer participants was anticipated with 30 farmers coming from each region. However, 254 farmers were able to participate in the survey, thus giving an average of 25 farmers from each region. This shortfall was mainly because there was a very poor response to the electronic questionnaires which were sent to farmers' electronic mail addresses provided by the Emerging Commercial Farmers Union of Namibia. Of the 150 respondents from Khomas and Omaheke regions who were recipients of the questionnaires, only 2 respondents completed the questionnaire. The questionnaires were sent to a five-fold increased sample size to achieve a 15-20% response rate as suggested by Boone and co-authors (2011) but unfortunately this did not improve the yield. It would have been ideal for the principal researcher to have delivered and administered the questionnaire, but this was not possible since commercial farms are sparsely located, which was going to be financially prohibitive and visiting by appointments was going to be time intensive as it is illegal to access such private properties unannounced.

On the other hand, most of the self-administered questionnaires were successfully completed with only a handful of farmers encountered who were illiterate and had none of their household members who could read and assist in completing the questionnaire. These farmers accounted for the small shortfall in the anticipated sample size in the communal areas. This could have been improved by recruitment of interpreters instead of self-

administered questionnaires, but this was not possible due to financial constraints, since 8 main local languages are spoken in Namibia and, moreover, they also come with local variations.

Communal farmers showed enthusiasm and a sizeable number were concerned about the effects of drought on their animals and willingly showed some of their cattle which were in the process of dying because of starvation. This calls for possible collaboration of farmers, the government and local authorities to come up with farmer mentorship programmes which teach farmers strategic animal management practices during adverse conditions to avoid livestock losses.

## **5.2 Overview of Bovine Tuberculosis risk factors**

There are many risk factors for exposure to BTB in cattle. Whilst most of them can be controlled, wildlife contact is a risk factor that remains a persistent challenge worldwide (Sergeant et al., 2017). Globally, BTB risk factors include disease prevalence in a country, regionally and across national boundaries, trans-border or local cattle movement, favourable survival conditions for pathogen, close interaction of cattle and people infected with tuberculosis caused by *M. bovis*, food consumption behaviour such as raw milk and lesioned meat consumption and lack of efficient disease surveillance, monitoring and eradication programmes (Friyantha, 2008; Fine et al., 2011; Napp et al., 2019).

Most herd level risk factors are applicable worldwide. The type of production such as intensive systems, geographical location and feed storage practices which may predispose to BTB through feed contamination by well-known reservoirs are most prevalent in the developed world (Garnett et al., 2002; Downs et al., 2018). In Namibia, the majority of the cattle are raised extensively on pasture or rangelands, whilst a minority are raised intensively in feedlots and dairy farms (Chitate et al., 2019). The dry climatic conditions in Namibia do not offer favourable conditions for prolonged *M. bovis* survival, therefore geographical location is a low risk factor. Namibia has a semi-arid low rainfall climate translating to poor pasture particularly in the dry winter season, consequently, cattle raised solely on pasture lose condition during this period. However, because the cattle are mostly indigenous breeds and therefore hardy, they tend to thrive in the same adverse conditions that have prevented exotic breeds from prospering. Namibian commercial farmers produce crossbred cattle using the *Bos indicus* breeds whereas most of the communal farmers raise the more hardy indigenous Nguni and Sanga cattle (Lepen, 1996).

At individual animal level, breed, age, parity and poor body condition score are risk factors for BTB particularly in areas where the disease is endemic which is not the case with Namibia since it is officially BTB free. However, these factors are interlinked with the livestock husbandry systems practiced which aim to maximize productivity (Cousins, 2001). Considering the extensive cattle production systems predominant in Namibia, individual animal level risks are low risk factors even if interlinked with production types.

### **5.3 Wildlife risk**

According to published reports wild animals remain the highest and most prevalent risk factor for BTB in cattle in Africa and the rest of the world (Friyantha, 2008). In this study, owning wildlife together with cattle has been found to be a growing trend in Namibia where there is increasing game farming to meet the demands of the venison market regionally and overseas (Lindsey, 2011; Lindsey et al., 2013). Game farming is being practiced by commercial farmers as evident in this study, whereby over a quarter of the commercial farmers owned a small number of wildlife.

Approximately half of the farmers participating in the survey responded to seeing wildlife on and around their premises, which is an indicator of the presence of wildlife as a high risk factor to almost half of the cattle population in Namibia. This result compares well with a similar investigation at a wildlife/livestock interface done in Ethiopia in which 19% of respondents reported wildlife-cattle interactions, however, 59% of farmers living close to a national park observed more encounters between wildlife and cattle by virtue of their geographical location (Tschopp et al., 2009). In a study done in South Africa by Sichewo and co- authors (2020), in corroboration with findings from this study, most farmers acknowledged sharing of resources between cattle and wildlife, which posed risk of transfer of pathogens, and the study area was also a wildlife/livestock/human interface. The findings from this study highlight the increased interaction between cattle and wildlife in Namibia, which is almost comparable with findings at wildlife/livestock interfaces where there is increased risk of exposure to BTB.

While just over a third of the farmers confirmed that their cattle shared grazing and a water source with wildlife, it can be assumed that all wildlife seen was most likely grazing and drinking along with their cattle. As these wild animals do not get tested for BTB before introduction into the game farms, this poses a risk of transmission of BTB through sharing stagnant water-sources and feeding on wet blades of grass. Furthermore, depending on the animal density within that area, aerosol transmission is possible. However, it has been

established that it is rare for *M. bovis* to be isolated from contaminated soil and pasture under field conditions, implying that environmental contamination is of little significance in the transmission of disease (Maddock, 1936). Other authors have supported the latter finding, suggesting the need for a high infective dose of *M. bovis* ( $10^7$ ) for oral transmission to be possible as opposed to a very low dose of one bacillus required for aerosol transmission (Neill et al., 1988; O'Reilly and Daborn, 1995). On game farms, as observed in this study, the animals are prone to a potentially contaminated environment which can allow the build-up of an infective dose sufficient for oral transmission to occur, therefore environmental transmission is significant because of continued opportunity of exposure. In a study done in South Africa, comparable results were obtained whereby 58% and 47% of participants observed their cattle drinking or sharing grazing with wildlife respectively and these participants had BTB positive herds, while BTB negative herds were associated with farmers whose cattle did not interact with wildlife (Sichewo et al., 2020). This shows that there is increased risk of aerosol transmission of BTB at close contact when cattle and wildlife congregate to drink and when they share pasture. The increased interaction of cattle and wildlife in the study in South Africa was mainly attributed to free access of livestock into game parks by wildlife officials in order for livestock to access better grazing, grazing of livestock adjacent to game reserves and the breakdown of game proof fences (Sichewo et al., 2020).

The wild animals observed in this study, namely warthogs, kudus, mongooses and meerkats have been documented to be able to carry and harbour BTB, moreover, these species cannot be contained by fences, which allows them to move freely, even increasing their risk of BTB infection and subsequent introduction into farms (Dejene et al., 2016). Therefore in the event of BTB infected wildlife, the majority of cattle in contact with these animals are at a high risk of exposure to infection. Wildlife observation frequency was high, increasing the chance of transmission of disease as it can be extrapolated that the more these wild animals are seen, the more likely are interactions with cattle, increasing the likelihood of transmission in aerosols, drinking water and grazing.

#### **5.4 Cattle to cattle interactions risks**

Proximity to international borders acts a risk factor for BTB cattle to cattle transmission across borders. Cattle can be moved illegally or can move freely across borders as was seen when cattle casually crossed the Kavango river into Angola and the Zambezi river into Zambia and vice versa. However, from the study it was observed that most farmers were at least 10 km from international borders. Nevertheless, this risk was considered high because

about 10% of the respondents had farms at most 10km from the border, therefore their cattle were likely to move across borders and be exposed to BTB. The study showed that about 20% of farmers had cattle moving at least 10km in search of grazing, which increased the likelihood of cattle located close to international borders crossing over to nearby countries. The cattle that may be encountered across the borders are of unknown BTB status, therefore are high risk animals particularly across Angola, Botswana and Zambia, where freedom from BTB has not been declared (Cosivi et al., 1995). In Zambia, BTB is one of the major infectious diseases of livestock whose prevalence and distribution remains unknown across the country (Malama et al., 2019).

Herd size was found to be a low risk factor for BTB as most farmers had small herd sizes, however, the risk became greater because of the possibility of interaction with other herds from farms with partial or absent fencing as they moved in search of grazing. Comparable results were also obtained in a study in Ethiopia whereby 81% of herd sizes were found to be small with less than 10 animals therefore posing as a very low risk factor of exposure to BTB, consequently, on tuberculin testing, these herds had very low reactivity (Tschopp et al., 2009). The fact that cattle walk large distances every day in search for grazing was found to be a moderate exposure factor to BTB, it also posed a risk of transmission across national boundaries for farms which were in very close proximity to the border with increased potential for interaction with foreign cattle of unknown BTB status.

Distance travelled by cattle in search for grazing carries an additional risk of the likelihood of encountering wildlife. Although distance moved by cattle in search of grazing was of moderate significance, this risk factor cannot be ignored because if such herds are exposed to BTB, they become sources of infection for other cattle herds.

The water-source for cattle in this study was found to be a low risk factor since only about a quarter of the farmers had their cattle drinking from rivers communally, which encourages close contact between cattle when they aggregate and transmission of BTB if there are infected cattle. While this is less likely in Namibia because it is officially BTB free, they may be increased risk of contact between cattle and wildlife at these uncontrolled communal water sources. Contrastingly, this risk was found to be significant in Western Uganda and in the United States of America, whereby cattle using uncontrolled water sources had an increased risk of BTB, as BTB is prevalent in these countries (Kazoora et al., 2014; Broughan et al, 2016). In a study done in Spain, it was found that although there were streams where animals could drink communally, these streams were dispersed, which discouraged aggregation of animals, hence having communal rivers or streams was a very

low risk factor for BTB (Cowie et al., 2014). Contrary to Spain, in which BTB is prevalent, Namibia has only a few rivers that survive drying out due to the persistent droughts, so animal aggregation is inevitable, but BTB transmission is likely to be low (Cowie et al., 2014).

## **5.5 Application of quarantine and BTB testing**

In most developed nations, BTB has been eradicated through national test and slaughter programmes and milk pasteurization (Tschopp, 2015). BTB control schemes include passive and active surveillance, strict animal movement control, mandatory quarantine and BTB testing of newly acquired animals and implementation of test-and-slaughter policies involving herd testing with the OIE recommended tuberculin skin test. Positive reactors are subsequently slaughtered, and retesting is done after prescribed periods until all reactors are eliminated (FAO, 2012).

The absence of cattle quarantine was a very high risk factor of exposure to BTB, with a 77.3% possibility of transmission of disease if one or some of the cattle were infected with any disease, particularly BTB. The study showed that quarantine was largely lacking in Namibia although it is of the utmost importance particularly in regions north of the veterinary cordon fence as well as those close to the borders to prevent infectious disease outbreaks, including FMD. Since BTB is a chronic disease with long incubation periods without overt clinical signs, the quarantine period allows for BTB testing and disposal of infected animals, therefore preventing disease introduction to other cattle herds. The practice of introducing new animals which may be infected into a BTB free herd is an acknowledged high risk factor for transmission of the disease in both industrialized and developing countries (Humblet et al., 2009). Transmission of BTB is mainly via aerosol, and a single infected animal if missed can spread disease in a herd over time. Furthermore, the process allows for observation of clinical symptoms of other diseases of significant importance such as FMD.

This study established that BTB testing of cattle herds was almost non-existent, with close to 70% of farmers indicating absence of testing. This posed a very high risk of BTB, because the true BTB status in cattle which can be only be ascertained through testing, remains unknown. Ever since Namibia started reporting freedom from BTB to the OIE, surveillance data based on BTB surveillance by meat inspection in export abattoirs, testing of weaner cattle destined for the South African market, dairy herds and game animals destined for export has been used to demonstrate freedom. The gap in BTB testing in the rest of the cattle population leaves a risk of transmission by cattle that may not go through post mortem

surveillance, and a reduced frequency of testing has been found to increase the prevalence and reduce the diagnosis of BTB (Kwaghe et al., 2015). Moreover, export abattoirs account for about 85% of all slaughter in Namibia, leaving a high risk of BTB in the unaccounted slaughtered animals (Chitate et al., 2019). A combination of BTB testing and meat inspection have been singled out as the best practices for BTB surveillance, therefore a lack of these may indicate weaknesses in the surveillance systems (EFSA, 2003). These high risk practices can have serious consequences, such as silent propagation of BTB in Namibia, potentially leading to loss of its official freedom status and subsequent loss of access to lucrative beef export markets.

In a retrospective study done in northern England, it was found that instead of farmers having their cattle tested for BTB annually according to the national regulations, most of the cattle were being tested for BTB once in 2 or 3 years (Ramírez-Villaescusa et al., 2010). Consequently, infected cattle increased and it was not known when they had become infected, which emphasizes the importance of regulated BTB testing in controlling the disease (Ramírez-Villaescusa et al., 2010). In the same study, there were no legal restrictions on movement of cattle and pre-movement testing for BTB, which allowed movement of infected cattle, thus spread of infection that could have been prevented had there been strict BTB control policies (Ramírez-Villaescusa et al., 2010).

Other countries in Africa lacking BTB control programmes include Zambia, Ethiopia and Tanzania and consequently, *M. bovis* is prevalent in these countries and prospects of eradication are remote (Katale et al., 2013; Dejene et al., 2016; Malama et al., 2019). In Zambia, neither the prevalence nor distribution of BTB is known (Katale et al., 2013; Dejene et al., 2016; Malama et al., 2019).

## **5.6 BTB awareness and zoonotic potential**

Wild animal proximity to humans seen in this study potentially exposed them not only to BTB if infected but other zoonotic pathogens as well. Ignorance about BTB was a high risk factor which could lead to not taking any safety precautions when dealing with cattle products and meat. This is of public health concern considering many potential zoonoses such as brucellosis, Q fever and listeriosis. Ignorance or a lack of disease awareness of farmers as observed in the study whereby almost 60% of respondents were unfamiliar with BTB could lead to consumption of abnormal carcasses posing a high risk of zoonoses and could also result in underreporting of disease and little motivation to seek veterinary services. The findings from the study were comparable to those observed in Tanzania in the Serengeti

ecosystem whereby about 65% of the farmers were completely unaware of BTB, which left them and their livestock vulnerable to exposure to BTB and other diseases (Katale et al., 2013). Results from a study done in South Africa also corroborate this study, whereby there was poor knowledge of BTB transmission and 56% of the households owning cattle were unaware of its zoonotic potential, which could lead to disregard of precautionary practices due to ignorance (Sichewo et al., 2019). Contrasting findings were observed in studies in Ethiopia, indicating that 70% of farmers were knowledgeable about BTB and its clinical signs either in cattle or in humans, and in South Africa, showing that most people at the wildlife/livestock/human interface under study were knowledgeable, attributed to prior field days and disease awareness campaigns that had been done in the study area (Tschopp et al., 2009; Sichewo et al., 2020). About 83% of commercial farmers in this study were knowledgeable about the disease attributable to a higher level of literacy and easier access to veterinary services. However, ignorance about BTB can be attributable to the fact that the disease is no longer a subject of discussion since its eradication in Namibia.

Consumption of milk as a predisposing factor for BTB infection was found to be very low. The majority of respondents taking milk took it soured and therefore were at a reduced risk of exposure, since *M. bovis* may survive in soured milk only if the bacterial load is very high (Michel et al., 2015). Despite these facts, it is also relevant to note that the age groups drinking milk were mostly children less than 12 years old, which potentially left them vulnerable to zoonoses since their immune systems may not yet be fully competent and since consumption was mostly on a daily basis so they were exposed to cumulative risk. Commercial farmers did not consume milk from their cattle so that their calves would achieve high weaning weights, thus increasing productivity. Contrasting findings were observed in Brazil, where 92% of respondents consumed milk and of these, 86.9% consumed it boiled (Belchior et al., 2016). However, on farms where milk was consumed raw, 5.8% of the cattle had tested positive for BTB which shows that raw milk consumption is a high risk practice, particularly in BTB prevalent countries such as Brazil (Belchior et al., 2016).

Absence of meat inspection at informal slaughter was identified as a very high risk factor. It further increased exposure to zoonoses, particularly BTB. With most of the cattle destined for local markets, including informal markets, some diseased carcasses could find their way into the unsuspecting consumer population, exposing them to diseases. In addition, at informal slaughter, BTB lesions are missed due to the absence of meat inspection which acts as one of the standard active surveillance methods for BTB implemented only at formal slaughter facilities in many countries including Namibia. From the study, of the cattle that

went through meat inspection, some were condemned because of abscesses, bovine measles, pneumonia (which may be part of BTB lesions) while some abnormalities were unknown, which echoes the importance of post mortem surveillance for all slaughter. Meat inspection facilitates the removal of contaminated organs and carcass condemnation of those with disseminated BTB lesions, therefore controlling the spread of *M. bovis* to humans and also facilitating trace back to the herd of origin (De la Rúa–Domenech, 2006). Other livestock such as goats and sheep that were slaughtered and consumed without going through meat inspection could also serve as portals for the transmission of zoonoses.

The presence of historical and current TB infections in households were medium risk factors, since the exact causative pathogen in these cases cannot be easily ascertained by getting a definitive diagnosis clarifying whether there is risk of transmission to cattle or vice versa (Kahler, 2015). It has been established that there is an underestimation and poor understanding of the impact of zoonotic TB on the global burden of TB because the tests used to diagnose human TB in most countries worldwide do not differentiate *M. bovis* from *M. tuberculosis* (Olea-Popelka et al., 2012). While transmission is most likely where there is increased proximity between cattle and people, particularly in rural areas where cattle kraals are within the homestead, the majority of Namibian cattle are raised extensively, thus less likely to be infected with BTB originating from humans.

The findings of this study showed lower human cases (12%) than those from a survey done in Ethiopia where 24% of households had a history of human TB cases in their households. In that study, these households were about a quarter of those whose cattle had reacted positively to the tuberculin skin test, suggesting likely anthrozoosis (Tschopp et al., 2009). This link was also found in a study done in Zambia by Cook and co-workers (1996), but, in both Ethiopia and Zambia, BTB is prevalent with likely transmission from cattle to humans, as compared to Namibia which is officially free from BTB (Cook et al., 1996). This association raises the need for further investigation of cattle linked to households with human TB cases in Namibia to see whether the link does exist, since human TB cases reported in this study were relatively high.

Table 5.1. Overview of risk levels found for risk factors investigated in the study

<b>Risk factor</b>	<b>Risk level</b>
Proximity to border	High
Cattle herd size	Low
Wildlife contact	Very high
Cattle to cattle interaction	High
Water source for cattle	Low
Distance moved by cattle searching for grazing	Medium
Lack of disease awareness	High
Application of BTB testing and quarantine	Very high
Absence of meat inspection at informal slaughter	Very high
Milk consumption behaviour	Very low
History of household human tuberculosis	Medium

## 5.7 Conclusion

The lack of an efficient BTB control scheme with its associated routine quarantine and BTB testing strategies are high risk factors of BTB in Namibia. However, wildlife contact is the most common high risk factor as it poses the greatest challenge for maintaining the BTB freedom status of Namibia. The long term potential consequences the wildlife risks carry are silent propagation of BTB in Namibia which may be difficult to eradicate leading to loss of its official freedom status and subsequent loss of market access and revenue from lucrative beef export markets.

## 5.8 Recommendations

The presence of wildlife in Namibia, which is a very high risk factor, necessitates a periodic 3 year cyclic testing for BTB in all cattle herds both north and south of the veterinary cordon fence as a method of active surveillance, as is being done in New Zealand with high levels of success in controlling BTB in cattle (Livingstone et al., 2015). Mandatory quarantine and BTB testing of cattle acquired locally should be done to control and prevent local disease transmission. Public awareness campaigns on diseases of public health concern and their role on the prevention and control of such diseases should be conducted regularly.

## REFERENCES

- ABAWI, K., 2013. Data collection instruments (questionnaire & interview). *Geneva: Geneva Foundation for Medical Education and Research.*
- AGRIFORUM, 2018 .Agriforum Namibia;<http://www.agriforum.com.na> [accessed on 12 June 2018]
- AMENI, G., VORDERMEIER, M., FIRDESSA, R., ASEFFA, A., HEWINSON, G., GORDON, S.V. & BERG, S., 2011. *Mycobacterium tuberculosis* infection in grazing cattle in central Ethiopia. *The Veterinary Journal*, 188(3), 359-361.
- AMENI, G., TADESSE, K., HAILU, E., DERESSE, Y., MEDHIN, G., ASEFFA, A., HEWINSON, G., VORDERMEIER, M. & BERG, S., 2013. Transmission of *Mycobacterium tuberculosis* between farmers and cattle in central Ethiopia. *PLoS One*, 8(10), p.e76891.
- ANDERSON, D.P., RAMSEY, D.S.L., NUGENT, G., BOSSON, M., LIVINGSTONE, P., MARTIN, P.A.J., SERGEANT, E., GORMLEY, A.M. AND WARBURTON, B., 2013. A novel approach to assess the probability of disease eradication from a wild-animal reservoir host. *Epidemiology & Infection*, 141(7), 1509-1521.
- ANON. (1994). - *Mycobacterium bovis* tuberculosis in animals and humans. CDR weekly, 4(3).
- ARANAZ, A., COUSINS, D., MATEOS, A. AND DOMÍNGUEZ, L., 2003. Elevation of *Mycobacterium tuberculosis* subsp. *caprae* Aranaz et al. 1999 to species rank as *Mycobacterium caprae* comb. nov., sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, 53(6), 1785-1789.
- ASHFORD, D., WHITNEY, E., RAGHUNATHAN, P. & COSIVI, O. 2001. Epidemiology of selected mycobacteria that infect humans and other animals. *Revue scientifique et technique (International Office of Epizootics)*, 20, 325-337.
- AWADA, L., TIZZANI, P., ERLACHER-VINDEL, E., FORCELLA, S. & CACERES, P. 2018. *Bovine tuberculosis: worldwide picture.*
- AYELE, W.Y., NEILL, S.D., ZINSSTAG, J., WEISS, M.G. AND PAVLIK, I., 2004. Bovine tuberculosis: an old disease but a new threat to Africa. *The International Journal of Tuberculosis and Lung Disease*, 8(8), 924-937.
- BELCHIOR, A.P.C., LOPES, L.B., GONÇALVES, V.S.P. AND LEITE, R.C., 2016. Prevalence and risk factors for bovine tuberculosis in Minas Gerais State, Brazil. *Tropical Animal Health and Production*, 48(2), 373-378.

- BOONE, W.J., TOWNSEND, J.S. AND STAVELAND, J., 2011. Using Rasch theory to guide the practice of survey development and survey data analysis in science education and to inform science reform efforts: An exemplar utilizing STEBI self-efficacy data. *Science Education*, 95(2), 258-280.
- BROUGHAN, J.M., MAYE, D., CARMODY, P., BRUNTON, L.A., ASHTON, A., WINT, W., ALEXANDER, N., NAYLOR, R., WARD, K., GOODCHILD, A.V. AND HINCHLIFFE, S., 2016. Farm characteristics and farmer perceptions associated with bovine tuberculosis incidents in areas of emerging endemic spread. *Preventive Veterinary Medicine*, 129, 88-98.
- BOYNTON, P.M. AND GREENHALGH, T., 2004. Selecting, designing, and developing your questionnaire. *Bmj*, 328(7451), 1312-1315.
- BUDDLE, B., SKINNER, M., WEDLOCK, D., COLLINS, D. & DE LISLE, G. 2002. New generation vaccines and delivery systems for control of bovine tuberculosis in cattle and wildlife. *Veterinary Immunology and Immunopathology*, 87, 177-185.
- CALEY, P., HICKLING, G.J., COWAN, P.E. AND PFEIFFER, D.U., 1999. Effects of sustained control of brushtail possums on levels of *Mycobacterium bovis* infection in cattle and brushtail possum populations from Hohotaka, New Zealand. *New Zealand Veterinary Journal*, 47(4), 133-142.
- CHAMBERS, M., GORDON, S., OLEA-POPELKA, F. AND BARROW, P. eds., 2018. *Bovine Tuberculosis*. CABI.
- CHEN, Y., CHAO, Y., DENG, Q., LIU, T., XIANG, J., CHEN, J., ZHOU, J., ZHAN, Z., KUANG, Y. & CAI, H. 2009. Potential challenges to the Stop TB Plan for humans in China; cattle maintain *M. bovis* and *M. tuberculosis*. *Tuberculosis*, 89, 95-100.
- CHIRIBOGA, L. M., KILMER, C., FAN, R. & GAWANDE, K. 2008. Does Namibia have a comparative advantage in beef production. *Bush School of Government and Public Service. Texas, United States*.
- CHITATE, F., FOSGATE, G.T. AND BOSHOFF, A. 2019. Namibia's demonstration of freedom from bovine tuberculosis.
- COMMISSION, I. O. O. E. B. S. & COMMITTEE, I. O. O. E. I. 2008. *Manual of diagnostic tests and vaccines for terrestrial animals: mammals, birds and bees*, Office international des épizooties.
- COOK, A.J.C., TUCHILI, L.M., BUVE, A., FOSTER, S.D., GODFREY-FAUSSETT, P., PANDEY, G.S. AND MCADAM, K.P.W.J., 1996. Human and bovine tuberculosis in the Monze District of Zambia—a cross-sectional study. *British Veterinary Journal*, 152(1), 37-46.
- COSIVI, O., MESLIN, F., DABORN, C. & GRANGE, J. 1995. Epidemiology of *Mycobacterium bovis* infection in animals and humans, with particular reference to

- Africa. *Revue Scientifique et Technique-Office International des Epizooties*, 14, 733-750
- COUSINS, D.V., 2001. Mycobacterium bovis infection and control in domestic livestock. *Revue Scientifique et Technique-Office International des Epizooties*, 20(1), 71-85.
- COWIE, C.E., HUTCHINGS, M.R., BARASONA, J.A., GORTÁZAR, C., VICENTE, J. AND WHITE, P.C., 2016. Interactions between four species in a complex wildlife: livestock disease community: implications for Mycobacterium bovis maintenance and transmission. *European Journal of Wildlife Research*, 62(1), 51-64.
- DEPARTMENT OF AGRICULTURE, FORESTRY AND FISHERIES (DAFF), Republic of South Africa. 2018. Interim bovine tuberculosis scheme manual (6 December 2018). <http://www.nda.agric.za/vetweb/pamphlets&Information/Policy/TB%20Manual%20%2006Dec2018%20interim%20signed.pdf> (Accessed 28 April 2019)
- DE GARINE-WICHATITSKY, M., CARON, A., KOCK, R., TSCHOPP, R., MUNYEME, M., HOFMEYR, M. & MICHEL, A. 2013. A review of bovine tuberculosis at the wildlife–livestock–human interface in sub-Saharan Africa. *Epidemiology & Infection*, 141, 1342-1356.
- DEJENE, S.W., HEITKÖNIG, I.M., PRINS, H.H., LEMMA, F.A., MEKONNEN, D.A., ALEMU, Z.E., KELKAY, T.Z. AND DE BOER, W.F., 2016. Risk factors for bovine tuberculosis (bTB) in cattle in Ethiopia. *PLoS One*, 11(7), p.e0159083.
- DE LA RUA-DOMENECH, R., GOODCHILD, A., VORDERMEIER, H., HEWINSON, R., CHRISTIANSEN, K. & CLIFTON-HADLEY, R. 2006. Ante mortem diagnosis of tuberculosis in cattle: a review of the tuberculin tests,  $\gamma$ -interferon assay and other ancillary diagnostic techniques. *Research in Veterinary Science*, 81, 190-210.
- DIRECTORATE VETERINARY SERVICES. 2011. Namibia Livestock Sector Strategy Final Report. Ministry of Agriculture, Water, Fisheries and Forestry, Windhoek, Republic of Namibia
- DOWNS, S. H., PARRY, J. E., UPTON, P. A., BROUGHAN, J. M., GOODCHILD, A. V., NUNEZ-GARCIA, J., GREINER, M., ABERNETHY, D. A., CAMERON, A. R., COOK, A. J., RUA-DOMENECH, R. D. L., GUNN, J., PRITCHARD, E., RHODES, S., ROLFE, S., SHARP, M., VORDERMEIER, H. M., WATSON, E., WELSH, M., WHELAN, A. O., WOOLLIAMS, J. A., MORE, S. J. & CLIFTON-HADLEY, R. S. 2018. Methodology and preliminary results of a systematic literature review of ante-mortem and post-mortem diagnostic tests for bovine tuberculosis. *Preventive Veterinary Medicine*, 153, 117-126.
- DURNEZ, L., SADIKI, H., KATAKWEBA, A., MACHANG'U, R. R., KAZWALA, R. R., LEIRS, H. & PORTAELS, F. 2009. The prevalence of Mycobacterium bovis-infection and

- atypical mycobacterioses in cattle in and around Morogoro, Tanzania. *Tropical Animal Health and Production*, 41, 1653-1659.
- EFSA PANEL ON BIOLOGICAL HAZARDS (BIOHAZ), 2013. Scientific Opinion on the public health hazards to be covered by inspection of meat (bovine animals). *EFSA Journal*, 11(6), 3266.
- EUROPEAN FOOD SAFETY AUTHORITY, 2003. *EFSA Journal*. EFSA.
- FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO) .2012. Animal Production and Health Division. Bovine tuberculosis at the animal-human-ecosystem interface. *Transboundary Animal Diseases Bulletin*. 40:1-11.
- FOOD AND AGRICULTURAL ORGANIZATION (FAO). 2012. Bovine tuberculosis at the animal interface. EMPRES: *Transboundary Animal Diseases Bulletin* 40:1–11. [www.fao.org/empres](http://www.fao.org/empres)
- FINE, A.E., BOLIN, C.A., GARDINER, J.C. AND KANEENE, J.B., 2018. A study of the persistence of *Mycobacterium bovis* in the environment under natural weather conditions in Michigan, USA. *Veterinary Medicine International*, 2011.
- FINE, A.E., O'BRIEN, D.J., WINTERSTEIN, S.R. AND KANEENE, J.B., 2011. An effort to isolate *Mycobacterium bovis* from environmental substrates during investigations of bovine tuberculosis transmission sites (cattle farms and wildlife areas) in Michigan, USA. *ISRN Veterinary Science*, 2011.
- FITZGERALD, S.D., KANEENE, J.B., 2012. Wildlife reservoirs of bovine tuberculosis worldwide: hosts, pathology, surveillance, and control. *Veterinary Pathology*. 50:488-499.
- FRIYANTHA, M. A. R. 2008. Overview: different strategies applied for control of bovine tuberculosis. *Tropical Agriculturist*, 157, 75-86.
- FOX, N.J., BARROW, P.A. AND HUTCHINGS, M.R., 2018. Role of wildlife in the epidemiology of *Mycobacterium bovis*. *Bovine Tuberculosis. CAB International*, 93.
- GARNETT, B.T., DELAHAY, R.J. AND ROPER, T.J., 2002. Use of cattle farm resources by badgers (*Meles meles*) and risk of bovine tuberculosis (*Mycobacterium bovis*) transmission to cattle. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 269(1499), 1487-1491.
- GORDEJO, F. R. & VERMEERSCH, J. 2006. Towards eradication of bovine tuberculosis in the European Union. *Veterinary Microbiology*, 112, 101-109.
- GOPAL, R., GOODCHILD, A., HEWINSON, G., DE LA RUA DOMENECH, R. AND CLIFTON-HADLEY, R., 2006. Introduction of bovine tuberculosis to north-east England by bought-in cattle. *Veterinary Record*, 159(9), 265-271.
- GOVERNMENT OF NAMIBIA. 2007. Communal Land Reform Act,' *Government Gazette of the Republic of Namibia*.

- HAYDON, D.T., CLEAVELAND, S., TAYLOR, L.H. AND LAURENSEN, M.K., 2002. Identifying reservoirs of infection: a conceptual and practical challenge. *Emerging Infectious Diseases*, 8(12), 1468-1473.
- HLOKWE, T.M., DE KLERK-LORIST, L.M. AND MICHEL, A.L., 2016. Wildlife on the move: a hidden tuberculosis threat to conservation areas and game farms through introduction of untested animals. *Journal of Wildlife Diseases*, 52(4), 837-843.
- HLOKWE, T.M., SAID, H. AND GCEBE, N., 2017. Mycobacterium tuberculosis infection in cattle from the Eastern Cape Province of South Africa. *BMC Veterinary Research*, 13(1), 299.
- HUMBLET, M.-F., BOSCHIROLI, M. L. & SAEGERMAN, C. 2009. Classification of worldwide bovine tuberculosis risk factors in cattle: a stratified approach. *Veterinary Research*, 40, 1-24.
- HUMBLET, M.F., MOYEN, J.L., BARDOUX, P., BOSCHIROLI, M.L. AND SAEGERMAN, C., 2011. The importance of awareness for veterinarians involved in cattle tuberculosis skin testing. *Transboundary and Emerging Diseases*, 58(6), 531-536.
- JACKSON, R., DE LISLE, G.W. AND MORRIS, R.S., 1995. A study of the environmental survival of Mycobacterium bovis on a farm in New Zealand. *New Zealand Veterinary Journal*, 43(7), 346-352.
- JAJERE, S.M., ATSANDA, N.N., BITRUS, A.A., HAMISU, T.M. AND GONI, M.D., 2018. Occurrence of bovine tuberculosis among cattle herds from nomadic peri-urban settlements and cattle slaughtered at the municipal abattoir of Bauchi, North-Eastern Nigeria. *Journal of Advanced Veterinary and Animal Research*, 5(1), 53-59.
- KAHLER, B., 2015. Does Mycobacterium bovis contribute to the case load of human tuberculosis in Namibia.
- KATALE, B.Z., MBUGI, E.V., KARIMURIBO, E.D., KEYYU, J.D., KENDALL, S., KIBIKI, G.S., GODFREY-FAUSSETT, P., MICHEL, A.L., KAZWALA, R.R., VAN HELDEN, P. AND MATEE, M.I., 2013. Prevalence and risk factors for infection of bovine tuberculosis in indigenous cattle in the Serengeti ecosystem, Tanzania. *BMC Veterinary Research*, 9(1), 267.
- KAZOORA, H.B., MAJALIJA, S., KIWANUKA, N. AND KANEENE, J.B., 2014. Prevalence of Mycobacterium bovis skin positivity and associated risk factors in cattle from Western Uganda. *Tropical Animal Health and Production*, 46(8), 1383-1390.
- KILJUNEN, 1981. Cattle farming regions of Namibia . The land and its people.
- KWAGHE, A. V., VAKURU, C. T., IWAR, V. N., NDAHI, M. D., ABUBAKAR, A. G. & EZE, E. 2015. Bovine tuberculosis: effects and challenges faced by developed and developing countries in the eradication process. *CAB Reviews*, 10, 1-20.
- LEPEN, 1996. Breed Characterisation studies in Namibia

- LINDSEY, P. 2011. *An analysis of game meat production and wildlife-based land uses on freehold land in Namibia: links with food security*, TRAFFIC East/Southern Africa.
- LINDSEY, P. A., HAVEMANN, C. P., LINES, R. M., PRICE, A., RETIEF, T., RHEBERGEN, T., VAN DER WAAL, C. & ROMANACH, S. 2013. Benefits of wildlife-based land uses on private lands in Namibia and limitations affecting their development. *Oryx*, 47, 41-53.
- LIVINGSTONE, P.G., HANCOX, N., NUGENT, G. AND DE LISLE, G.W., 2015. Toward eradication: the effect of *Mycobacterium bovis* infection in wildlife on the evolution and future direction of bovine tuberculosis management in New Zealand. *New Zealand Veterinary Journal*, 63(sup1), 4-18.
- MAAS, M., MICHEL, A.L. AND RUTTEN, V.P., 2013. Facts and dilemmas in diagnosis of tuberculosis in wildlife. *Comparative Immunology, Microbiology and Infectious Diseases*, 36(3), 269-285.
- MACHADO, A., RITO, T., GHEBREMICHAEL, S., MUHATE, N., MAXHUZA, G., MACUAMULE, C., MOIANE, I., MACUCULE, B., MARRANANGUMBE, A.S., BAPTISTA, J. AND MANGUELE, J., 2018. Genetic diversity and potential routes of transmission of *Mycobacterium bovis* in Mozambique. *PLoS Neglected Tropical Diseases*, 12(1), p.e0006147.
- MADDOCK, E.C.G., 1933. Studies on the survival time of the bovine tubercle bacillus in soil, soil and dung, in dung and on grass, with experiments on the preliminary treatment of infected organic matter and the cultivation of the organism. *Epidemiology & Infection*, 33(1), 103-117.
- MAGWEDERE, K., HEMBERGER, M. Y., HOFFMAN, L. C. & DZIVA, F. 2012. Zoonoses: a potential obstacle to the growing wildlife industry of Namibia. *Infection Ecology & Epidemiology*, 2, 18365.
- MALAMA, S., MUNYEME, M. AND MUMA, J.B., 2019. Bovine Tuberculosis in Zambia. In *Tuberculosis in Animals: An African Perspective* (pp. 445-453). Springer, Cham.
- MARTÍN-HERNANDO, M.P., HÖFLE, U., VICENTE, J., RUIZ-FONS, F., VIDAL, D., BARRAL, M., GARRIDO, J.M., DE LA FUENTE, J. AND GORTAZAR, C., 2007. Lesions associated with *Mycobacterium tuberculosis* complex infection in the European wild boar. *Tuberculosis*, 87(4), 360-367.
- MEAT BOARD OF NAMIBIA .2009. Annual Report. Windhoek, Namibia.
- MENZIES, F.D. AND NEILL, S.D., 2000. Cattle-to-cattle transmission of bovine tuberculosis. *The Veterinary Journal*, 160(2), 92-106.
- MESINGER G. & SCHMEIDER J. (1989). - Rindertuberkulose. In *Tiergesundheitsjahrbuch der DDR 1989* (S. Kautzsch, ed.). Institut für angewandte Tierhygiene, Eberswalde-Finow, German Democratic Republic, 81-87.

- MICHEL, A.L., GEOGHEGAN, C., HLOKWE, T., RASELEKA, K., GETZ, W.M. AND MARCOTTY, T., 2015. Longevity of *Mycobacterium bovis* in raw and traditional souring milk as a function of storage temperature and dose. *PloS One*, 10(6), p.e0129926.
- MOGOTSI, K., KGOSIKOMA, O. E. & LUBINDA, K. F. 2016. Wildlife-livestock interface, veterinary cordon fence damage, lack of protection zones, livestock theft and owner apathy: Complex socio-ecological dynamics in Foot and Mouth disease control in southern Africa. *Pastoralism*, 6, 21.
- MUGAMBI, J.M., OMWENGA, S.G., WESONGA, H.O., MBATHA, P., GATHOGO, S., CHOTA, A.C., MAGWISHA, H.B., MAKONDO, Z.E., RUKAMBILE, E. AND MWAKAPUJA, R., 2016. Bovine Tuberculosis in East Africa. *African Crop Science Journal*, 24(1), 53-61.
- MÜLLER, B., DÜRR, S., ALONSO, S., HATTENDORF, J., LAISSE, C. J., PARSONS, S. D., VAN HELDEN, P. D. & ZINSSTAG, J. 2013. Zoonotic *Mycobacterium bovis*-induced tuberculosis in humans. *Emerging infectious diseases*, 19, 899.
- MUSOKE, J., HLOKWE, T., MARCOTTY, T., DU PLESSIS, B.J. AND MICHEL, A.L., 2015. Spillover of *Mycobacterium bovis* from wildlife to livestock, South Africa. *Emerging Infectious Diseases*, 21(3), 448.
- NARANJO, V., ACEVEDO-WHITEHOUSE, K., VICENTE, J., GORTAZAR, C. AND DE LA FUENTE, J., 2008. Influence of methylmalonyl-CoA mutase alleles on resistance to bovine tuberculosis in the European wild boar (*Sus scrofa*). *Animal Genetics*, 39(3), 316-320.
- NAPP, S., CIARAVINO, G., DE VAL, B.P., CASAL, J., SAÉZ, J.L. AND ALBA, A., 2019. Evaluation of the effectiveness of the surveillance system for tuberculosis in cattle in Spain. *Preventive Veterinary Medicine*, 173, p.104805.
- NEILL, S.D., POLLOCK, J.M., BRYSON, D.B. AND HANNA, J., 1994. Pathogenesis of *Mycobacterium bovis* infection in cattle. *Veterinary Microbiology*, 40(1-2), 41-52.
- NEILL, S.D., HANNA, J., O'BRIEN, J.J. AND MCCRACKEN, R.M., 1988. Excretion of *Mycobacterium bovis* by experimentally infected cattle. *The Veterinary Record*, 123(13), 340.
- OLEA-POPELKA, F., FREEMAN, Z., WHITE, P., COSTELLO, E., O'KEEFFE, J. 2012. Relative effectiveness of Irish factories in the surveillance of slaughtered cattle for visible lesions of tuberculosis, 2005–2007. *Irish Veterinary Journal* 65, 2.
- OIE, 2018, Chapter 3.4.6. Bovine tuberculosis. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 1058-1074, <https://www.oie.int> [accessed 20 March 2019]
- OIE, 2015. World animal health information data base interface. Paris: OIE, 2015. Available at: <<http://www.oie.int/wahis/public.php?page=home>>. [Accessed 8 May 2018]

- OFFICE INTERNATIONAL DES EPIZOOTIES (OIE); WORLD ORGANISATION FOR ANIMAL HEALTH. 2011. De-tailed country[ies] disease incidence: Bovine tuberculosis, period from 2005 to 2011. [www.web.oie.int/wahis/public.php?](http://www.web.oie.int/wahis/public.php?)[accessed 20 May 2018].
- OMER, M.K., SKJERVE, E., WOLDEHIWET, Z. AND HOLSTAD, G., 2001. A cross-sectional study of bovine tuberculosis in dairy farms in Asmara, Eritrea. *Tropical Animal Health and Production*, 33(4), 295-303.
- O'REILLY, L.M. AND DABORN, C.J., 1995. The epidemiology of *Mycobacterium bovis* infections in animals and man: a review. *Tubercle and Lung disease*, 76, 1-46.
- ORGANIZATION, W. H. 2009. *Global tuberculosis control: epidemiology, strategy, financing: WHO report 2009*, World Health Organization.
- PUCKEN, V.B., KNUBBEN-SCHWEIZER, G., DÖPFER, D., GROLL, A., HAFNER-MARX, A., HÖRMANSDORFER, S., SAUTER-LOUIS, C., STRAUBINGER, R.K., ZIMMERMANN, P. AND HARTNACK, S., 2017. Evaluating diagnostic tests for bovine tuberculosis in the southern part of Germany: a latent class analysis. *PLoS One*, 12(6), p.e0179847.
- RAMÍREZ-VILLAESCUSA, A.M., MEDLEY, G.F., MASON, S. AND GREEN, L.E., 2010. Risk factors for herd breakdown with bovine tuberculosis in 148 cattle herds in the south west of England. *Preventive Veterinary Medicine*, 95(3-4), 224-230.
- REILLY, L. & COURTENAY, O. 2007. Husbandry practices, badger sett density and habitat composition as risk factors for transient and persistent bovine tuberculosis on UK cattle farms. *Preventive veterinary medicine*, 80, 129-142.
- RENWICK, A., WHITE, P. & BENGIS, R. 2007. Bovine tuberculosis in southern African wildlife: a multi-species host-pathogen system. *Epidemiology & Infection*, 135, 529-540.
- ROMERO, B., RODRÍGUEZ, S., BEZOS, J., DÍAZ, R., COPANO, M.F., MEREDIZ, I., MÍNGUEZ, O., MARQUÉS, S., PALACIOS, J.J., DE VIEDMA, D.G. AND SÁEZ, J.L., 2011. Humans as source of *Mycobacterium tuberculosis* infection in cattle, Spain. *Emerging infectious diseases*, 17(12), 2393.
- RUTTEN, V. & MICHEL, A. L. Tuberculosis in Wildlife, "a risk of *M. bovis* infection for livestock and a hindrance for *M. avium* subsp. *paratuberculosis* vaccination". Proceedings of the 10th Berlin-Brandenburgischer Rindertag, Oktober 2014, 2014. CUVILLIER VERLAG, Göttingen 2014, 76-79.
- SALTZ, D., WARD, D., KAPOFI, I. & KARAMATA, J. 2004. Population estimation and harvesting potential for game in arid Namibia. *South African Journal of Wildlife Research-24-month Delayed Open Access*, 34, 153-161.

- SIBHAT, B., ASMARE, K., DEMISSIE, K., AYELET, G., MAMO, G. AND AMENI, G., 2017. Bovine tuberculosis in Ethiopia: a systematic review and meta-analysis. *Preventive Veterinary Medicine*, 147, 149-157.
- SKUCE, R.A., ALLEN, A.R. AND MCDOWELL, S.W., 2012. Herd-level risk factors for bovine tuberculosis: a literature review. *Veterinary Medicine International*, 2012.
- SKUCE, R.A., BYRNE, A.W., LAHUERTA-MARIN, A. AND ALLEN, A., 2018. 5 Mycobacterium bovis Molecular Typing and Surveillance. *Bovine Tuberculosis*, 58.
- SERGEANT, E.S.G., HAPPOLD, J. AND LANGSTAFF, I., 2017. Evaluation of Australian surveillance for freedom from bovine tuberculosis. *Australian Veterinary Journal*, 95(12), 474-479.
- SICHEWO, P.R., ETTER, E.M. AND MICHEL, A.L., 2020. Wildlife-cattle interactions emerge as drivers of bovine tuberculosis in traditionally farmed cattle. *Preventive Veterinary Medicine*, 174, 104847.
- SICHEWO, P.R., MICHEL, A.L., MUSOKE, J. AND ETTER, E., 2019. Risk factors for zoonotic tuberculosis at the wildlife–livestock–human Interface in South Africa. *Pathogens*, 8(3), 101.
- SICHEWO, P.R., VANDER KELEN, C., THYS, S. AND MICHEL, A.L., 2020. Risk practices for bovine tuberculosis transmission to cattle and livestock farming communities living at wildlife-livestock-human interface in northern KwaZulu Natal, South Africa. *PLOS Neglected Tropical Diseases*, 14(3), p.e0007618.
- SMITH, N.H., BERG, S., DALE, J., ALLEN, A., RODRIGUEZ, S., ROMERO, B., MATOS, F., GHEBREMICHAEL, S., KAROUI, C., DONATI, C. AND DA CONCEICAO MACHADO, A., 2011. European 1: a globally important clonal complex of Mycobacterium bovis. *Infection, Genetics and Evolution*, 11(6), 1340-1351.
- SOLIMAN, Y.A., GHONIM, M., NASR, E.A., MOUSSE, I., EBIARY, E.A. AND SELIM, S.A., 2004. Cloning and expression of recombinant MPB70 protein antigen from Mycobacterium bovis BCG for diagnosis of tuberculosis. *The Egyptian Journal of Immunology*, 11(2), 21-29.
- STRYDOM, P. & MUSELER, D. L. 1996. The performance of the Namibian beef industry. *Change*, 1, 95.
- SULIEMAN, M.S. AND HAMID, M.E., 2002. Identification of acid fast bacteria from caseous lesions in cattle in Sudan. *Journal of Veterinary Medicine, Series B*, 49(9), pp.415-418.
- TSCHOPP, R., 2015. 15 Bovine Tuberculosis at the Human–Livestock–Wildlife Interface in Sub-Saharan Africa. *One Health: The Theory and Practice of Integrated Health Approaches*, 163.

- TSCHOPP, R., SCHELLING, E., HATTENDORF, J., ASEFFA, A. AND ZINSSTAG, J., 2009. Risk factors of bovine tuberculosis in cattle in rural livestock production systems of Ethiopia. *Preventive Veterinary Medicine*, 89(3-4), 205-211.
- VORDERMEIER, H. M., HUYGEN, K., SINGH, M., HEWINSON, R. G. & XING, Z. 2006. Immune responses induced in cattle by vaccination with a recombinant adenovirus expressing Mycobacterial antigen 85A and *Mycobacterium bovis* BCG. *Infection and immunity*, 74, 1416-1418.
- VORDERMEIER, M., AMENI, G., BERG, S., BISHOP, R., ROBERTSON, B. D., ASEFFA, A., HEWINSON, R. G. & YOUNG, D. B. 2012. The influence of cattle breed on susceptibility to bovine tuberculosis in Ethiopia. *Comparative Immunology, Microbiology and Infectious Diseases*, 35, 227-232.
- WALSH, A., MORGAN, D., PHE, T., DAVIDSON, J., ANDERSON, L., ZENNER, D., THOMAS, L., TAMNE, S., ABUBAKER, I. AND PHE, T., 2008. Bovine tuberculosis: Guidance on management of the public. *Personnel*, 15, 19.
- WHITNEY, H.G., 2017. The eradication of bovine tuberculosis in Canada. *The Canadian Veterinary Journal*, 58(8), 859.
- WHO, 2012. Global tuberculosis report 2012. Available at: ([http://www.stop.org/wg/news\\_vaccines/](http://www.stop.org/wg/news_vaccines/))
- WORLD HEALTH ORGANIZATION, 2010, November. The Control of Neglected Zoonotic Diseases: Community Based Interventions for NZDs Prevention and Control. In *Report of the Third Conference Organized with ICONZ, DFID-RIU, SOS, EU, TDR and FAO with the Participation of ILRI and OIE (23-24)*.
- WORLD HEALTH ORGANIZATION, 2009. WHO report: global tuberculosis control: epidemiology, strategy, financing. *Geneva, Switzerland: WHO*, 1-9.
- WOODROFFE, R., DONNELLY, C.A., JOHNSTON, W.T., BOURNE, F.J., CHEESEMAN, C.L., CLIFTON-HADLEY, R.S., COX, D.R., GETTINBY, G., HEWINSON, R.G., LE FEVRE, A.M. AND MCINERNEY, J.P., 2005. Spatial association of *Mycobacterium bovis* infection in cattle and badgers *Meles meles*. *Journal of Applied Ecology*, 42(5), 852-862.
- WRAY, C., 1975. Survival and spread of pathogenic bacteria of veterinary importance within the environment. *Veterinary Bulletin*.
- <https://www.tbfacts.org/bovine-tb/> [accessed 10 May 2018]

# APPENDICES

## Appendix 1. Ministry of Health and Social Services of Namibia Research Division approval



### REPUBLIC OF NAMIBIA

#### Ministry of Health and Social Services

Private Bag 13190  
Windhoek  
Namibia

Ministerial Building  
Harvey Street  
Windhoek

Tel: 061 - 203 2507  
Fax: 061 - 222 558  
E-mail: itaship087@gmail.com

#### OFFICE OF THE EXECUTIVE DIRECTOR

Ref: 17/3/3 RM  
Enquiries: Mr. A. Shipanga

Date: 15 August 2019

Dr. Blessing Muza  
PO Box 9  
Tsumeb  
Namibia

Dear Dr. Muza

**Re: An investigation of Bovine Tuberculosis (BTB) Risk Factors in the cattle population in Namibia**

1. Reference is made to your application to conduct the above-mentioned study.
2. The proposal has been evaluated and found to have merit.
3. **Kindly be informed that permission to conduct the study has been granted under the following conditions:**
  - 3.1 The data to be collected must only be used for academic purposes;
  - 3.2 No other data should be collected other than the data stated in the proposal;
  - 3.3 Stipulated ethical considerations in the protocol related to the protection of Human Subjects should be observed and adhered to, any violation thereof will lead to termination of the study at any stage;

AS

Appendix 2. (V014-19) UP Faculty of Veterinary Science Animal Ethics Committee approval



Faculty of Veterinary Science  
Animal Ethics Committee

11 October 2019

Approval Certificate  
New Application

AEC Reference No.: V014-19  
Title: AN INVESTIGATION OF BOVINE TUBERCULOSIS RISK FACTORS IN THE NAMIBIAN CATTLE POPULATION  
Researcher: Dr BF Muza  
Student's Supervisor: Prof AL Michel  
Dear Dr BF Muza,

The **New Application** as supported by documents received between and for your research, was approved by the Animal Ethics Committee on its quorate meeting of.

Please note the following about your ethics approval:

1. The use of the following is approved:  
**Questionnaire designed and administered with the main aim to investigate BTB risk factors in Namibia.**
2. Ethics Approval is valid for 1 year and needs to be renewed annually by.
3. Please remember to use your protocol number (V014-19) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.  
Yours sincerely

A handwritten signature in black ink, appearing to read 'V Naidoo'.

Prof V Naidoo  
CHAIRMAN: UP-Animal Ethics Committee

Room 013, Avon Park Building, Oosloepoed  
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Fakulteit Veeartseniekunde  
Lefapha la Diseense tsa Bongakadirulwa

Appendix 3. (V014-19) UP Faculty of Humanities Research Ethics Committee approval



12 March 2020

Dear Dr BF Muza

**Project Title:** AN INVESTIGATION OF BOVINE TUBERCULOSIS RISK FACTORS IN THE NAMIBIAN CATTLE POPULATION  
**Researcher:** Dr BF Muza  
**Supervisor:** Prof AL Michel  
**Department:** Veterinary Tropical Diseases  
**Reference number:** 18379738 (V014-19)  
**Degree:** Masters

I have pleasure in informing you that the above application was **approved** by the Research Ethics Committee on 12 March 2020. Data collection may therefore commence.

Please note that this approval is based on the assumption that the research will be carried out along the lines laid out in the proposal. Should the actual research depart significantly from the proposed research, it will be necessary to apply for a new research approval and ethical clearance.

We wish you success with the project.

Sincerely,

A handwritten signature in blue ink, appearing to read 'Innocent Pikirayi'.

**Prof Innocent Pikirayi**  
**Deputy Dean: Postgraduate Studies and Research Ethics**  
**Faculty of Humanities**  
**UNIVERSITY OF PRETORIA**  
**e-mail: PGHumanities@up.ac.za**

Fakulteit Geesteswetenskappe  
Lefapha la Bomotheo

Research Ethics Committee Members: Prof I Pikirayi (Deputy Dean); Prof KL Harris; Mr A Bious; Dr A-M de Beer; Dr A dus Santos; Ms KT Govindar; Andrew; Dr P Gutuza; Dr E Johnson; Prof D Maseko; Mr A Mohamed; Dr I Nkomé; Dr C Rutter; Prof D Reyburn; Prof M Soes; Prof E Tsaloni; Prof V Thebe; Ms B Tsaba; Ms D Nickalapa

## Appendix 4 (REC 126-20) UP Faculty of Veterinary Science REC Approval



Faculty of Veterinary Science

Research Ethics Committee

04 November 2020

### LETTER OF APPROVAL

**Ethics Reference No** REC126-20  
**Protocol Title** AN INVESTIGATION OF BOVINE TUBERCULOSIS RISK FACTORS IN THE NAMIBIAN CATTLE POPULATION  
**Principal Investigator** Dr BF Muza  
**Supervisors** Prof AL Michel

Dear Dr BF Muza,

We are pleased to inform you that your submission conforms to the requirements of the Faculty of Veterinary Sciences Research Ethics committee.

Please note the following about your ethics approval:

1. Please use your reference number (REC126-20) on any documents or correspondence with the Research Ethics Committee regarding your research.
2. Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
3. Please note that ethical approval is granted for the duration of the research as stipulated in the original application (for Post graduate studies e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
4. The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

1. 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.
2. **Applications using Animals:** FVS ethics recommendation does not imply that AEC approval is granted. The application has been pre-screened and recommended for review by the AEC. Research may not proceed until AEC approval is granted.

NOTES: Approved. This application was submitted retrospectively; it was conducted with AEC (V014-19) and Humanities approval (Ref nr: 18379738).

We wish you the best with your research.

Yours sincerely

**PROF. M. OOSTHUIZEN**  
Chairperson: Research Ethics Committee

**100**  
YEARS  
OF EXCELLENCE

Private Bag 2010, Hatfield, Pretoria  
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Faculty of Veterinary Science  
Fakulteit Veterinsykunde  
Letapha la Disensente eka Mangakaditruswa

## Appendix 5. Letter of invitation to survey participant



### DEPARTMENT OF VETERINARY TROPICAL ANIMAL DISEASES

Dear Participant,

#### **Questionnaire for MSc research on Bovine Tuberculosis (BTB)**

I am a veterinarian undertaking an MSc (Tropical Animal Health) degree through the University of Pretoria, South Africa and the Institute of Tropical Medicine, Antwerp. As I am a veterinarian living and working in Namibia I chose to do a research on BTB as this is an economically important disease. BTB was eradicated in 1985 in Namibia but known and published risk factors of exposure to BTB are in existence in Namibia though they have not been officially investigated and assessed. I want to qualitatively assess the risk factors of BTB in the Namibian cattle population so I intend to seek for answers from farmers through the use of a structured questionnaire.

Cattle production is the biggest financial contributor in the agricultural sector in Namibia from which most farmers derive their livelihood hence the freedom status from BTB is of invaluable importance to trade which the nation strives to maintain through control and surveillance methods. Despite these activities, BTB risk factors have to be known as this will allow a risk based surveillance strategy which is more cost effective for the Ministry of Agriculture, Water and Forestry. If need be, depending on outcome of research, recommendations can be made on better control strategies and policies to maintain the BTB freedom status in Namibia thus benefiting the whole nation. A Master of Science dissertation and degree for postgraduate studies will be achieved.

Attached is a structured questionnaire, it would be greatly appreciated if you could complete it and send it back in the return envelope enclosed or email the completed questionnaire to the address ([muzablessing89@gmail.com](mailto:muzablessing89@gmail.com)) and if hand delivered, may you hand it over to the deliverer upon completion. It should take no longer than twenty minutes to complete. If you would prefer a paper copy, please do not hesitate to contact me and I shall send one by post. Your confidentiality will be protected and no responses will be linked to you personally in the publication of the results. If you wish to receive an electronic copy of the thesis once it has been accepted for publication, please indicate this at the end of the questionnaire.

Thank you very much for your assistance, it is greatly appreciated.

Kind regards,

Researcher:

Blessing

Muza

Supervisor: Prof. Anita Michel (Department of Veterinary Tropical Diseases +2721 529 8269)

Appendix 6. Consent form given to farmers consenting to participation in the survey

**QUESTIONNAIRE**

**Informed consent**

Information that you provide while answering this questionnaire will only be used for the purposes of this project only. The results will be used for the investigator's MSc thesis.

All questionnaires to be used as well as the information derived from the answered questionnaires will be compiled, coded in Microsoft excel , filed electronically and stored in a computer and separate external hard drive as a backup, this information is confidential so hardcopies will be stored in a safe for safekeeping .Hardcopies and any personal information held will be destroyed once the study has been concluded. No personal information will be disseminated in any written or oral publication or presentation.

Participation in this questionnaire is voluntary and you are able to withdraw from answering further questions or abstaining from answering certain questions at any time.

**Signature:** .....

Appendix 7. The questionnaire used as a data collection tool for the project which was translated into local languages



## QUESTIONNAIRE SURVEY

QSN500001

**INSTRUCTIONS: CIRCLE NUMBER() (ROMAN NUMERAL) CORRESPONDING TO APPROPRIATE RESPONSE, WHERE APPLICABLE ADD A COMMENT TO PROVIDE FURTHER DETAILS**

### SECTION 1: FARM DEMOGRAPHICS

1. **(a)** In which farming region is your farm located?

- (i) Oshikoto (v) Ohangwena (ix) Zambezi  
(ii) Khomas (vi) Omaheke (x) Kavango  
(iii) Ozondtjupa (vii) Omusati  
(iv) Kunene (viii) Oshana

**(b)** Which country are you closest to, within 200km?

- (i) Botswana (iii) South Africa (v) none  
(ii) Angola (iv) Zambia

**(c)** How far away from the border is your farm?

- (i) less than 5km (iii) 11-20km (v) 51-100km  
(ii) 5-10km (iv) 21-50km (vi) more than 100km

2. Type of farming (If both apply circle both options)

- (i) Commercial (ii) Communal

3. What is your herd size including cows, heifers, bulls, oxen, calves?

- (i) 5 - 20 (iii) 50 - 99 (v) above 500  
(ii) 21 - 49 (iv) 100 - 499

4. **a)** Do you own any other livestock species ?

- (i) yes (ii) no

**b)** If yes which other livestock species do you own?

- (i) goats (iii) pigs  
(ii) sheep (iv) others namely .....

5. (a) Apart from livestock do you also own wildlife?

- (i) yes (ii) no

(b) Do you see wild animals on your premises?

- (i) yes (ii) no

## SECTION 2:FARMING PRACTICES

6. Is the area where your cattle graze fenced off from neighbouring cattle herds?

- (i) yes (ii) no (iii) partially

7. Are your cattle grazing with other farmers' cattle on the same pasture?

- (i) yes (ii) no

8. Where do your cattle drink water?

- (i) river (ii) dam  
(iii) pumped water in a trough (iv) man made water hole

9. a) Do wild animals share the grazing area with your cattle?

- (i) yes (ii)no

(b) Do wild animals share a water source with your cattle?

- (i) yes (ii) no

(c) If yes which species namely?

- (i) kudu (iii) wildebeest (v) others namely.....

- (ii) meerkats (iv) warthogs (vi) mongooses

(d) How often do you see them together with your cattle?

- (i) everyday (iii)once a week (v)less than once every fortnight  
(ii) twice a week (iv)once in a fortnight

10. How far is the distance your herd moves at most in search of grazing?

- (i) less than 5km (iii) between 10-20km  
(ii) between 5 -10km (iv) more than 20km

11. How many cattle did you sell last year on hoof and or directly for slaughter?

- (i) 1- 10 (iii) 21-50  
(ii) 11- 20 (iv) above 50

12. To whom do you sell your cattle?

- (i) South African market (iii) Meatco/Haartlief  
(ii)Local market (iv) Uncertain

13. (a)How many cattle did you buy last year?

- (i)none (iv )21-50  
(ii) less than 10 (v) more than 50  
(iii) 10-20

(b) How many cattle did you receive/acquire last year (as gifts)?

- (i)none (iii )21-50  
(ii) less than 10 (iv) more than 50  
(ii) 10-20

(c) Are the cattle placed in quarantine/isolation after purchase?

- (i)Yes (ii) no

### SECTION 3: DISEASE AWARENESS

14. When last were your cattle tested for Bovine tuberculosis?

- (i) never (iv) 3 years ago
- (ii) 1 year or less ago (v) more than 3 years ago
- (iii) 2 years ago

15. Are you familiar with the disease called Bovine tuberculosis?

- (i) yes (iii) no

16. Can Bovine tuberculosis also affect humans?

- (i) yes (ii) no (iii) not sure

17. (a) If you sold cattle to a commercial abattoir in the past, were any of your cattle/parts/organs not pass during meat inspection?

- (i) yes (ii) no (iii) uncertain

(b) If yes what was the reason?

- (i) Abscesses (iii) Lumpy skin disease (v) Pneumonia (vii) Unknown
- (ii) Contagious abortion (iv) Bovine measles (vi) Other namely.....

18. (a) Do you and/or any members of your household drink milk from your cattle?

- (i) Daily (iii) Never/Rarely
- (ii) Twice a week (iv) Once per week

(b) If yes, who consumes the milk from your cattle?

- (i) Children < 12 years of age (iii) Adults 18 to 65 years of age
- (ii) Children 12 – 18 years of age (iv) Adults above 65 years of age

(c) If yes in which form do you consume it? (Tick all options that are applicable)

- (i) raw (ii) boiled (iii) soured

19. Do you slaughter any of the following species for home consumption? (Indicate option/s applicable)

(a) cattle ? (i) yes (ii) no

(b) goats? (i) yes (ii) no

(c) sheep? (i) yes (ii) no

20 How often do your slaughter animals for home consumption?

(a) Cattle?

- (i) Once per month (iii) Once per every 6 months (v) Never/rarely
- (ii) Once every 3 months (iv) Once to twice per year

(b) Goats

- (i) Once per month (iii) Once per every 6 months (v) Never/rarely
- (ii) Once every 3 months (iv) Once to twice per year

(c) Sheep

- (i) Once per month (iii) Once per every 6 months (v) Never/rarely
- (ii) Once every 3 months (iv) Once to twice per year

21 Is the meat inspected by a veterinary official?

- (i) Yes (ii) no

22 If you observe an abnormal appearance of any of the internal organs of the slaughtered bovine, what do you do with it?

- (i) Ignore and prepare as for normal organs
- (ii) Cut out the abnormally looking part of the organ and discard it in the veld
- (iii) Cut out the abnormally looking part of the organ and burn it
- (iv) Cut out the abnormally looking part of the organ and bury it

(v) Cut out the abnormally looking part of the organ and feed it to the dogs

(vi) Burn that organ

(vii) Take the abnormal organs to a veterinarian

**23 (a)** Do you have members of your household or employees who are currently being treated for human tuberculosis?

(i) yes

(ii)no

**(b)** Did anyone in your household or working for you in the past have a history of human tuberculosis?

(i) yes

(ii)no

**THE END**

**THANK YOU FOR YOUR PARTICIPATION!**

Appendix 8. Summary of results recorded in Microsoft excel

	KHOMAS	OMAHAK	KUNENE	OSHIKO	OSHANA	ZAMBEZI	OHANGW	OMUSATI	OTJOZO	KAVANG	TOTAL	
<b>1bi</b>	1	12				15			7		35	<b>BOTS</b>
ii			7	20	30		23	27	1	22	130	<b>ANGOLA</b>
iii									1	4	5	<b>SA</b>
iv						20				2	22	<b>ZAMB</b>
v	9	1	15	9			3		20		57	<b>NONE</b>
<b>ci</b>					1	4	4			7	16	<b>&lt;5KM</b>
ii					1	5	3			4	13	<b>5-10KM</b>
iii					5	7	5	1		4	22	<b>11-20KM</b>
iv		3	1	2	2	7	9	7		5	36	<b>21-50KM</b>
v		5		5	3	4	1	8		5	31	<b>51-100KM</b>
vi	1	5	6	16	18	3	1	11	11	2	74	<b>&gt;100KM</b>
<b>2i</b>	10	5	6	8	5	1	1		27	9	72	<b>COMMER</b>
ii		8	24	22	25	27	23	27	3	19	178	<b>COMMUN</b>
<b>3i</b>		1	16	17	30	10	15	26		19	134	<b>HERD 5-20</b>
ii		5	7	4		8	7	1	2	2	36	<b>21-49</b>
iii		2	3	5		7	3		14	2	36	<b>50-99</b>
iv	7	3	3	2		3	1		14	3	36	<b>100-499</b>
v	3	2	1	1		1				1	9	<b>&gt;500</b>
<b>4ai</b>	2	11	16	17	24	18	16	18	29	21	172	<b>YES- OTHER LIVESTOCK</b>
ii	8	2	14	12	6	12	7	7	1	5	74	<b>NO OTHER LIVESTOCK</b>
<b>bi</b>	2	8	14	16	25	16	19	17	20	20	157	<b>GOATS</b>
ii	5	4	3	6		2			15	1	36	<b>SHEEP</b>
iii		1	1	1		1	1	2	2	1	10	<b>PIGS</b>
iv										1	1	<b>OTHERS NAMELY</b>
<b>5ai</b>	5	1	2	3	1	6	2		8	7	35	<b>YES-OWN WILDLIFE</b>
ii	5	12	28	26	25	24	22	25	22	21	210	<b>NOT -OWN WILDLIFE</b>
<b>bi</b>	10	10	17	13	19	16	5	4	24	8	126	<b>YES-SEE WILDLIFE</b>
ii		3	13	17	12	14	17	21	6	17	120	<b>NOT - SEE WILDLIFE</b>
<b>6i</b>	10	8	21	21	26	6	15	6	28	8	149	<b>YES- FENCED OFF</b>
ii		3	5	6	1	21	8	14	2	15	75	<b>NOT FENCED OFF</b>
iii		2	3	4	2	2	1	5		5	24	<b>PARTLY FENCED OFF</b>
<b>7i</b>		6	8	10	1	24	12	13	2	23	99	<b>YES-GRAZING WITH CATTLE</b>
ii	10	7	22	20	29	6	12	12	28	5	151	<b>NOT GRAZING WITH OTHER</b>
<b>8i</b>		4	12	3	7	20	5	7		8	66	<b>RIVER-DRI NK WATER</b>
ii	1	2	1	5		4	1	2	6	17	39	<b>DAM -DRINK WATER</b>
iii	3	2	17	18	1	5	9	9	21	1	86	<b>PUMPED WATER</b>
iv	6	5		4	21	3	9	7	3	2	60	<b>MAN MADE WATERHOLE</b>
<b>9ai</b>	2	3	6	9		19	6	3	16	9	73	<b>YES- GRAZE WILDANIMALS</b>
ii	8	10	24	19	30	9	18	22	13	19	172	<b>NO-GRAZE WILDANIMALS</b>
<b>bi</b>	7	6	13	10	5	20	4	2	13	5	85	<b>YES- WATER WILDANMLS</b>
ii	3	7	17	18	25	10	20	23	17	23	163	<b>NO-WATER WLDANMLS</b>
<b>ci</b>	5	4	6	5		12	3		8	5	48	<b>KUDUS</b>
ii	1	1	3			2	2	2	4	7	22	<b>MEERCATS</b>
iii		1				8				1	10	<b>WILDEBBEST</b>
iv	5	5	7	6	13	4	1		10		51	<b>WARTHOGS</b>
v			2	3					1	4	10	<b>OTHERS NAMELY</b>
vi			1	1			1			5	8	<b>MONGOOSES</b>
<b>di</b>	4	4	2	4		2	1	2	6	7	32	<b>EVERYDAY</b>
ii	3	2	2	3		9	1		2	2	24	<b>TWICE A WEEK</b>

iii	1		6	1		7	1	1	6	3	26	ONCE A WEEK
iv		1		2	9	4			2	4	22	ONCE A FORTNIGHT
v		2	4	1	7	2	2		3	6	27	LESS THAN ONCE A FORTNIGHT
10i		3	18	13	16	13	13	18	10	7	111	<5KM SEARCH FOR GRAZING
ii	5	6	9	11	11	11	7	5	11	9	85	5-10KM
iii	3	3	2	5	1	3	1		8	5	31	10-20KM
iv	2		1	1	1	2	3	1	1	6	18	>20KM
11i		8	24	21	14	22	14	15	10	22	150	1-10 CATTLE SOLD
ii		1	1			3			5	1	11	11-20 CATTLE SOLD
iii		1		2					5		8	21-50 CATTLE SOLD
iv	10	3	3	3			1		10		30	>50 CATTLE SOLD
12i	8	4	6	1					9		28	SA MARKET
ii	1	6	11	16	11	23	11	6	12	13	110	LOCAL MARKET
iii	4	1	5	2	1				16	5	34	MEATCO/HEARTLIEF
iv		3	6	6	2	2	1	6		5	31	UNCERTAIN
13ai		6	24	23	29	21	19	19	9	9	159	NO CATTLE BOUGHT
ii	6	6	5	4	1	8	3	5	16		54	>10 CATTLE BOUGHT
iii	4	1	1	2			1		2		11	10-20 CATTLE BOUGHT
iv				1					3		4	21-50 BOUGHT
v								1			1	>50 CATTLE BOUGHT
bi	10	13	29	29	29	21	22	20	29	12	214	NONE RECEIVED
ii			1	1	1	7	1	5	1	13	30	<10 CATTLE RECEIVED
iii						1				1	2	10-20 CATTLE RECEIVED
iv							1				1	21-50 CATTLE RECEIVED
ci	10	6	2			9	5	5	4	12	53	YES-QUARANTINE
ii		7	28	29	30	19	16	17	25	14	185	NO-QUARANTINE
14i	1	8	26	22	28	23	17	16	21	8	170	NEVER- BTB TESTING
ii				4		4	3	4	2	11	28	<1 YR AGO-BTB TESTING
iii	1					1		2	1	3	8	2 YRS AGO-BTB TESTING
iv	2	1	4				4	1	1	2	15	3 YRS AGO- BTB TESTING
v	6	4		4	2	1		1	4	4	26	>3YRS AGO- BTB TESTING
15i	10	6	23	14	17	6	5	4	15	9	109	YES- FAMILIAR WITH BTB
ii		6	7	16	13	23	19	20	15	17	136	NOT- FAMILIAR WITH BTB
16i	7	3	13	15	14	8	7	3	13	13	96	YES-BTB AFFECTS HUMANS
ii	2	4	12	4	9	3	7	8	6	5	60	NO- BTB AFFECTS HUMANS
iii	1	5	5	10	7	16	10	12	11	10	87	NOT SURE- BTB IN HUMANS
17ai	3	2	3	4	3	2	3	3	8	9	40	YES- CONDEMED
ii	7	9	15	19	27	17	11	19	15	7	146	NOT-CONDEMNED
iii			8	3		10	7	2	7	10	47	UNCERTAIN IF CONDEMNED
bi	1	2	1	1		1		1	1	2	10	ABSCESS
ii							1			1	2	CA
iii	1			1			1	1	2	7	13	LSD
iv			1	1	1				1	1	5	BOVINE MEASLES
v	1			1	1	1				3	7	PNEUMONIA
vi							1			3	4	OTHERS NAMELY
vii				1	1	1	3	4	2	6	18	UNKNOWN
18ai		4	2	2		12	3	1	3	5	32	DAILY-DRINK MILK
ii		2		1	1	7	3	2		8	24	TWICE A WK-DRINK MILK
iii	9	5	26	25	24	5	17	21	24	10	166	NEVER/RARELY-DRINK MILK
iv		2	2	2	5	5	2	1	2	2	23	ONCE PER WK- DRINK MILK

<b>bi</b>		7	12	7	25	16	16	15	9	7	114	CHDR<12 YRS	
ii		4	8	6	11	16	14	6	5	7	77	CHDRN 12-18YRS	
iii		4	5	4	11	19	13	7	11	12	86	ADULTS 18-65 YRS	
iv		3	5	3	2	10	8	3	4	2	40	ADULTS>65 YRS	
<b>ci</b>		3	2	3	2	14	7	4	4		39	RAW	
ii		4	7	8	11	10	3	4	7	9	63	BOILED	
iii		5	13	7	20	14	12	11	11	8	101	SOURD	
<b>19ai</b>	10	11	26	20	18	23	11	11	27	15	172	YES-SL CATTLE	
ii		1	3	3	12	6	9	9	1	4	48	NOT- SLA CATTLE	
<b>bi</b>	2	9	11	11	17	10	19	13	20	16	128	YES- SLAUGHTER GOATS	
ii			1	1	6	4	2	8		2	24	NOT- SLAUGHTER GOATS	
<b>ci</b>	6	5	5	3		2	2	2	14	1	40	YES- SLA SHEEP	
ii				1		3	3	2		6	15	NO- SLAUGHTER SHEEP	
<b>20ai</b>			1	2			3	1		3	10	ONE A MNTH CATTLE	
ii	6		2	6		3	1	1	3	2	24	ONCE IN 3 MNTHS	
iii	2	2	2	4		3	3	1	11	6	34	ONCE IN 6 MNTHS	
iv	2	6	3	3	1	11	3	5	8	6	48	1-2 X A YR	
v		4	21	12	16	9	11	7	7	4	91	NEVER/RARELY CATTLE	
<b>bi</b>	1	1	2	3		1	3	1	2	8	22	ONCE PER MONTH	
ii	1	2	3	3		4	2		9	2	26	ONCE IN 3 MNTHS	
iii	1	5	1	4		3	1	3	6	4	28	ONCE IN 6 MNTHS	
iv		2	4	4	8	2	7	4	3	4	38	1-2 X A YR GOATS SLAUGH	
v			2		10	4	6	6		2	30	NEVER/RARE GOATS	
<b>ci</b>	1		2	2				1	2	8	16	ONCE A MNTH SHEEP SLAUGHT	
ii	1	2	2	2		1			7	1	16	ONCE EVERY 3 MNTHS SHEEP	
iii	2	1		1					4	1	9	ONCE IN 6MNTHS SHEEP	
iv	1		1	2		1	1	1	2	2	11	1-2 X A YR SHEEP SLAUGHTER	
v						2	3	4	2	7	18	NEVER/RARE SHEEP SLAUGHTER	
<b>21i</b>				1		3	7	3	1	9	24	YES- MEAT INSPECTION	
ii	10	11	30	28	29	25	18	21	28	17	217	NO- MEAT INSPECTION	
<b>22i</b>							4	1	2		7	IGNOREAND PREPARE AS NORM	
ii		1	5	3	16	8	6	10	3	2	54	CUT DISCARD IN VELD	
iii		1	1	2	1	1	1	3	1	9	20	CUT AND BURN	
iv	1	1	8	9	6	2	1		8	10	46	CUT AND BURY	
v		4	2	3	3	3	6	2	2	1	26	CUT FEED TO DOGS	
vi	2	4	4	3			2	5	1	3	24	BURN ORGAN	
vii	8	2	10	9	2	13	4	4	12	5	69	TAKE TO VET	
<b>23ai</b>			3		1	4	2	5	4	13	32	YES-CURRENT TX TB	
ii	10	13	27	29	27	25	23	20	27	13	214	NO-CURRENT TX TB	
<b>bi</b>	2	3	7	6	2	3	7	1	3	10	44	YES-HX TB	
ii	8	10	23	23	27	26	18	23	27	16	201	NO-HX TB	