

# **The prevalence of bovine tuberculosis and associated risk factors for humans in Swaziland**

By

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## List of Abbreviations

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BTB	Bovine Tuberculosis
CIST	Comparative Intra-dermal Skin Test
CMI	Cell mediated Immunity
ELISA	Enzyme Linked Immunosorbent Assay
IFN- $\gamma$	Interferon gamma
IYSIS	Inyoni Yami Swaziland Irrigation Scheme
<i>M. bovis</i>	<i>Mycobacterium bovis</i>
NVL	No Visible Lesion
OD	Optical density
OD-av	Optical density reading for avian tuberculin PPD stimulated plasma
OD-bov	Optical density reading for bovine tuberculin PPD stimulated plasma
OD-fort	Optical density reading for fortuitum PPD stimulated plasma
PCR	Polymerase Chain Reaction
PPD	Purified Protein Derivative
SMI	Swaziland Meat Industries
SNL	Swazi Nation Land (Communal Land)
TB	Tuberculosis

## Thesis Summary

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Title	<b>The prevalence of bovine tuberculosis and associated risk factors for humans in Swaziland</b>
By	<b>Mcebo Edwin Maswati Dlamini</b>
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Bovine Tuberculosis is a chronic debilitating disease of cattle and other animals with a worldwide distribution and transmitted mainly through the inhalation of aerosols. The aim of this study was to determine the prevalence of BTB in the cattle population of selected dip tanks in Swaziland. Furthermore, the zoonotic risk to farmers whose cattle are infected with BTB was assessed by means of a questionnaire survey. Abattoir surveillance identified 16 dip tanks of study where at least 10 % of the cattle were tested for BTB using the comparative intra-dermal skin. In five of these dip tanks, the same cattle were tested for BTB using the IFN- $\gamma$  Test. Eight BTB skin test positive animals were slaughtered and a detailed *post mortem* examination was conducted and samples collected for the isolation of *M. bovis*. Concurrent with BTB testing, a questionnaire survey was conducted to determine risk factors for humans. The prevalence of BTB was found to be 6.75 % in the study population and 20 % of BTB positive animals were diagnosed by both the CIST and IFN- $\gamma$ , indicating a correlation for the test positive animals in the two tests. *M. bovis* was isolated from seven of the eight animals slaughtered. Farmers' knowledge of BTB as a cattle disease and serious zoonosis is insufficient and inadequate while consumption practices of products of bovine origin exposes them to the risk of infection by *M. bovis*. There is a need to investigate the extent of *M. bovis* infections in the human population.

# Chapter 1

## Justification

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### 1.1 Literature review

#### 1.1.1 Introduction

##### Definition

Bovine tuberculosis (BTB) is a chronic insidious contagious disease primarily affecting cattle, as well as other wild and domestic ruminants, characterized by the progressive development of tubercles in tissues and organs of the body, with resultant caseation and calcification. The disease is thought not to be indigenous to Africa's free ranging mammals and the causative organism, *Mycobacterium bovis* is considered to be an alien organism within African ecosystems (Hofmeyr *et al.*, 2006).

##### Mycobacteria of domestic animals

There are several types of pathogenic Mycobacteria which affect domestic animals and human beings, such as the human type (*M. tuberculosis*) which primarily infects humans but also animals, the bovine type (*M. bovis*) which infects animals and man, *M. avium* which belongs to the *Mycobacterium avium* complex and the vole bacillus which infects murines (Wadhwa *et al.*, 2006). The *M. tuberculosis* complex further comprises *M. pinnipedii*, *M. caprae*, *M. canettii*, *M. africanum* and the oryx bacillus (Brosch *et al.*, 2002).

##### Hosts

BTB practically affects all species of vertebrates including mammals (Arega *et al.*, 2013). Cattle are considered to be the main hosts of *M. bovis* but the disease is also found in other species including humans, domesticated animals such as pigs and wildlife such as the African buffalo (Cousins, 2001). All species and all age groups are susceptible to tuberculosis with cattle, goats and pigs being the most susceptible and sheep and horse showing a high natural resistance (Wadhwa *et al.*, 2006). A breed susceptibility in BTB infections in cattle has been reported where *Bos indicus*, breeds such as Zebu and Brahman maybe more resistant to the disease compared to *Bos taurus* breeds such as Holsteins (Cousins, 2011). In some studies, not only was the prevalence of BTB found to be higher in Holsteins than in either crosses or Zebu cattle kept under identical husbandry conditions but also an increased disease severity was observed in the former (Ameni *et al.*, 2008). In addition, the researchers found that the risk

of BTB being found in Holsteins was more than twice than that in Zebu cattle (Ameni *et al.*, 2007). Subsequent studies in the variability of BTB in *Bos indicus* (Zebu) and *Bos taurus* cattle (Holsteins) indicated that the latter are more susceptible to BTB than the former (Cousins, 2011). Furthermore, there is a higher tuberculin skin test prevalence and IFN- $\gamma$  response to mycobacterial antigens that were observed in *Bos taurus* than in *Bos indicus* which could be due to differences in antigen recognition profiles between the two breeds (Ameni, 2012). Ameni *et al.* (2006) attributed this observed differences in the IFN- $\gamma$  between the two breeds to either the different BoLA alleles affecting the recognition of mycobacterial agents or to the fact that a high proportion of Holstein cattle in Ethiopia suffer from a far advanced disease than Zebu. The observed differences may also be due to multiple parasitic infections as these could modulate the IFN- $\gamma$  response to Mycobacteria antigens, as studies have shown that infection with either *Fasciola* spp or *Strongylus* spp significantly reduced skin indurations in response to bovine PPD in *M. bovis* infected heifers compared to *M. bovis* infected that had been dewormed (Ameni *et al.*, 2006).

Based on findings of recent epidemiological and immunological studies conducted in Ethiopia on host susceptibility differences between native Zebu cattle and exotic Holstein-Friesian cattle, the native Zebu cattle were found to be more resistant to BTB and *M. bovis* infection than the Holstein-Friesian cattle (Vordermeier *et al.*, 2006).

While all age group are susceptible to BTB, animals between the ages 5-9 years are at a higher risk of being BTB positive than those two years or younger (Ameni *et al.*, 2007). This is in line with the findings of Regassa *et al.* (2010) which states that the prevalence of BTB varies amongst age groups and management systems. While Ameni *et al.* (2007) had placed five years as the cut-off point for age, Regassa *et al.* (2010), found a slightly lower cut-off point for the age, stating that cattle over four years and with poor management systems are more likely to be tuberculin reactors than animals younger than four years and with good management systems (Regassa *et al.*, 2010).

In African buffaloes and cattle, the risk to contract *M. bovis* and consequently, BTB, increases with increasing age of the animal and more adult African buffaloes and cattle are affected than calves (Imitiaz *et al.*, 2008). This is due to the fact that in the presence of the infectious agent in the environment, older animals have been exposed for a longer period compared to younger animals.

While BTB as a disease may be considered as a single-host, single-pathogen interaction, *M. bovis*, just like a number of other pathogens, may simultaneously interact with multi-species in an ecosystem (Renwick *et al.*, 2007). This has a huge implication on the dynamics of the disease as factors such as the density of each susceptible host in the ecosystem, the ratio of inter-species transmission rate to intra-species transmission rates as well as the interaction rates between the hosts species, interact at various levels and intensities (Renwick *et al.*, 2007).

The wide host range of *M. bovis* indicate that it is a generalist pathogen, with a very low pathogen host specificity, making it to pose a greater threat to its hosts than it would have had it been a specialized pathogen with a high pathogen host specificity (Renwick *et al.*, 2007). The basic reproductive rate of *M. bovis*,  $R_0$ , being the average number of successful secondary infections produced when one infected individual is introduced into a susceptible population, is greater than 1,  $R_0 \geq 1$ , as infected animals remain infectious until they die, representing a continued source of infection for a number of years (Renwick *et al.*, 2007).

### **BTB in African Buffaloes**

The African buffalo (*Syncerus caffer*) is highly gregarious and a susceptible species to BTB with patterns of lesion development suggesting an aerosol transmission, making the animal an ideal maintenance host in the Southern African ecosystem (Renwick *et al.*, 2007). The disease manifests itself as a chronic and predominantly subclinical disease and is progressive within the herd (De Vos *et al.*, 2001). Buffaloes develop lesions in the lymph nodes of the head, tonsils, lung or thoracic lymph nodes within 3-6 months of infection, with the infection spreading by local expansion, via the blood or lymph vessels to more distal sites (Renwick *et al.*, 2007). *Post mortem* examinations of diseased animals reveal poor encapsulated lung lesions, which indicate a weak immune response, suggesting that the Southern African buffalo maybe a recent evolutionary host with a naive immunity (Renwick *et al.*, 2007). A further progression of lesions frequently results in caseous necrosis, followed by cavitation and liquefaction, at which stage the host becomes super infective (Renwick *et al.*, 2007).

Adult buffalo remain infected for about 3-5 years before mortality, with calves and yearling less likely to become infected but once infected, the diseases developing at a much faster rate to mortality (Renwick *et al.*, 2007).

BTB affects population growth, resilience and fecundity in buffalo herds (Renwick *et al.*, 2007). The high BTB prevalence observed in some herds is due almost entirely to intra-specific transmission as buffaloes in the immediate social group of those infected receive high and possibly multiple exposure to the disease, which may vary depending in the severity of lesions in the individual (Renwick *et al.*, 2007).

Environmental conditions such as rainfall affect the behavior of the buffalo, and in turn influence the prevalence of the disease (Renwick *et al.*, 2007). There is no sexual bias in buffalo in the vulnerability of BTB but an age related increase in prevalence has been observed (Renwick *et al.*, 2007). Calf survival rate is affected by BTB in buffaloes, as infected mother buffaloes are usually in poor body condition score at calving, resulting in decreased milk production and subsequent poor nutrition for the calf (Renwick *et al.*, 2007).

There is variation in the vulnerability of buffaloes to BTB as the pathogenesis of the disease is dependent on the animals' genetic resistance, condition and nutrition status, with the latter two factors fluctuating with environmental variables such as rainfall, grazing, temperature and UV light exposure (Renwick *et al.*, 2007). Poor body condition increases the risk to infection by *M. bovis* and subsequent disease development and BTB has been demonstrated to be in synergy with parasitism and resource limitation to significantly decrease the body condition score in buffaloes (Renwick *et al.*, 2007).

Garine-Wichatitsky *et al.* (2010) observed that an increase in BTB prevalence amongst buffaloes in the Kruger National Park was associated with a decrease in overall body condition, with animals in herds with a BTB prevalence being found to be in a poor body condition and also losing body condition faster than those in herds with zero or medium prevalence. These animals in herds with a high BTB prevalence also exhibited the greatest decline in body condition and a corresponding increase in fecal egg counts (Garine-Wichatitsky *et al.*, 2010).

It was found that the prevalence of BTB was the highest in high milk yielding water buffaloes ( $P < 0.015$ ) and in higher parity African buffaloes (Imitiaz *et al.*, 2008). This was thought to be due to the fact that high yield and pregnancy are the key factors which aggravate the disease load and animals with these factors are immune-compromised due to long term production stress (Imitiaz *et al.*, 2008).

## **BTB in Greater Kudu**

The kudu is another gregarious herbivores and BTB presents with severe abscessation of the head lymph nodes with draining sinus tracts on the parotid area of the head, with the infectious exudates contaminating leaves and thorns on vegetation (Renwick *et al.*, 2007). Infection is transmitted to other grazing kudu who would be feeding on contaminated vegetation especially during the dry season when the animals compete to browse on palatable thorn trees such as *Acacia* spp and *Xiziphus* spp (Renwick *et al.*, 2007).

Due to the kudu's diet of *Acacia* leaves in thorn trees, the animal usually has micro-scars in the pharyngeal and oesophageal mucous membranes which serve as a port of entry for *M. bovis* and localising in adjacent predilection sites such as the tonsils and the retropharyngeal, mandibular and cervical lymph nodes, before the infection spreads to other distal sites such as the lungs and abdominal organs (Renwick *et al.*, 2007).

Clinical signs associated with BTB in the kudu are emaciation, coughing, blindness, a swollen head and neck, as well as draining sinus tracts, with the latter two enabling experienced veterinarian and other practitioners to tentatively diagnose BTB in the kudu from a distance (Renwick *et al.*, 2007).

While the common buffalo strain of *M. bovis* is responsible for BTB in the kudu, a different strain of the *Mycobacteria* has been isolated in the Kruger National Park, indicating the possibility of a separate infection cycle other than the dominant strain of infections, which has implications for the potential role of the kudu as a maintenance host (Michel *et al.*, 2009).

## **BTB in Baboons**

While primates are susceptible to BTB, and usually infected by inhalation, the disease is rarely found in free ranging animals, with infections found in troops of baboons in the Kruger National Park attributed to scavenging by the animals on infected carcasses (Renwick *et al.*, 2007). The most common clinical signs of BTB in baboons are emaciation, coughing and dyspnoea, with depression observed in some infected animals (Renwick *et al.*, 2007). On *post mortem* examination, miliary lesions of the lungs and spleen are observed, indicating a rapid spread of the infection through the blood streams. Extensive lesions are also found in the kidney, liver, vertebrae and the mesenteric and peripheral lymph nodes (Renwick *et al.*, 2007). Baboons and primates in general are spill-over hosts or dead-end hosts and BTB cannot be maintained in the species without an external source of infection (Renwick *et al.*, 2007).

## BTB in Carnivores

Lions, cheetahs and leopards are some of the carnivores that are susceptible to BTB, with restriction fragment length polymorphism analysis confirming that the *M. bovis* strains found in lions in the Kruger National Park were the same as that found in buffaloes, providing evidence of prey to predator transmission in carnivorous animals (Michel *et al.*, 2009). As predators, carnivores prey on weakened animals and/or dead animals, raising the prospect of feeding on BTB infected material such as lungs and lymph nodes, and subsequently being infected in the process. While most carnivores get infected while feeding, the ingestion of muscle tissue alone poses minimum risk as *M. bovis* does not readily multiply in meat, although selective feeding is not common amongst predators (Renwick *et al.*, 2007). Transmission of infection from prey to predator may also occur via the respiratory route during terminal bite asphyxiation of infected prey (Renwick *et al.*, 2007). Furthermore, lions, such as those found in the Kruger National Park, the Serengeti grasslands, the woodlands in Northern Tanzania and Southern Kenya, continuously infect each other through biting and aerosol transmission (Bakalar, 2005).

In lions infected with BTB, clinical signs observed include emaciation, staring hair-coat with poorly healing skin lesions, swollen joints and limbs, lameness and blindness. On necropsy, well developed lesions are found in the lymphatic system but the lesions show no signs of caseation or calcification and their macro-appearance is unlike lesions found in primates and ruminants (Renwick *et al.*, 2007).

Besides mortality, BTB has a devastating effect on the social behavior of lions, as infected males are weakened by the chronic disease, leading to a faster territorial male turnover and consequent infanticide, eviction of entire prides and a decrease in average longevity (Bakalar, 2005).

## Distribution

BTB occurs worldwide (**Figure 1**) with eradication programs in progress or near completion in a number of countries in Europe, the United States, Canada, Japan and New Zealand (Wadhwa *et al.*, 2006). Some countries such as Australia, Denmark, Sweden and Finland are considered to be free of BTB (Wadhwa *et al.*, 2006). The disease is more prevalent in most parts of Africa, Asia and the Americas (Renwick *et al.*, 2007). Many developed countries have either reduced or eliminated BTB from their cattle populations even though significant pockets of infection still remain in wildlife in Canada, the United Kingdom, the United States, New Zealand and Europe (Renwick *et al.*, 2007).

In Africa, BTB is present in a majority of countries, although there is a strong regional difference in the number of outbreaks, cases and deaths (Renwick *et al.*, 2007). While that may be the case, only seven countries apply BTB disease control measures and consider it to be a notifiable diseases (Renwick *et al.*, 2007). Some African countries have never reported the disease to the OIE while other countries previously reported the presence of the disease but have not done so in recent years (Renwick *et al.*, 2007). In other African countries, the suspected cases of BTB are prevalent while in other countries, the presence of clinical disease has been confirmed (**Figure 1**).

The struggle by developing countries to control and eradicate BTB may be due to a number of factors which may include lack of financial resources to meet the high cost of a sustainable testing program, social unrest due to political instability, ethnic wars, displacement of large numbers of human and animal populations, lack of veterinary expertise and communication networks, insufficient collaboration with bordering countries and hence lack of effective quarantine and the smuggling of cattle across state boundary (Ameni *et al.*, 2007).

The disease may persist in a population for a long time before it is detected. Human beings are also susceptible to BTB and hence it may be a very important zoonosis, with significant public health concerns, and significant socio-economic effects, especially in third world countries. Transmission in humans is mainly through the drinking of unpasteurized milk from infected cows, directly via respiratory aerosols of cattle or from the consumption of meat from infected slaughtered bovines (Huchzermeyer *et al.*, 1994). In rural and/or semi urban areas of developing countries, the bulk of the milk produced is not pasteurized and hence a lot of milk is consumed unpasteurized. Veterinary services responsible for meat inspection are also not widely available, resulting in a lot of meat, including high risk viscera, being consumed without *post mortem* inspection. Hence, in the presence of infected livestock, a large number of people are at risk of exposure to *M. bovis*. Coupled with inadequate disease management and reporting it renders BTB a public health problem in underdeveloped and developing countries (Imitiaz *et al.*, 2009).

In the Kruger National Park, South Africa, bovine tuberculosis was first discovered in 1990 in African buffaloes (*Syncerus caffer*) and not only does it have implications for the conservation of wildlife species, but also for the health of humans and livestock living at the wildlife-livestock-human interface (Michel, 2012). In 2008, *M. bovis* was isolated from African buffaloes in Zimbabwe, and the pathogen was suspected to have crossed the Limpopo River from the Kruger National Park to Zimbabwe (Garine-Wichatitsky *et al.*, 2010). Further studies revealed evidence of an epidemiologic link between the *M. bovis* isolated in buffaloes from the

Gonarezhou National Park in Zimbabwe and the Kruger National Park in South Africa, although there is still a need to accurately determine the genetic relationship between *M. bovis* isolates from the two National Parks (Garine-Wichatisky *et al.*, 2010). Should this be confirmed, it will give evidence to the transboundary spread of BTB (Garine-Wichatisky *et al.*, 2010).

While studies have confirmed the presence of BTB in animal populations of countries such as Egypt, Nigeria and the DRC, a recent (2006) study in Tanzania found that 88 % of villages screened had at least one animal positive for BTB ([www.clubofmozambique.com](http://www.clubofmozambique.com)).

In Southern Africa, and even to the rest of Africa, the more recent detection of other potential maintenance hosts indicate that BTB exists as a multi-hosts pathogen within a multi-species system (Renwick *et al.*, 2007). The relatively high number of interacting large mammal species in the vast savannah regions of Africa is relatively higher than in any other geographic area of the world of similar size, favouring the rapid spread of BTB through this ecosystem (Renwick *et al.*, 2007).

### **BTB prevalence in selected countries and regions**

In 106 cattle herds in the Kafue basin of Zambia, using the comparative intra-dermal skin test, Munyeme *et al.* (2008), found the herd prevalence of BTB to be 49.8 %. In 2009, in the livestock/wildlife interface area of Lochinvar and Blue lagoon, as well as Kuzungula, an area outside the livestock/wildlife interface, all in Zambia, the overall prevalence of BTB in 944 heads of cattle from 111 herds was found to be 6.8 % but the BTB prevalence in the cattle at Lonchivar, Blue Lagoon and Kuzungula was 5.2 %, 9.6 % and 0.8 % respectively (Munyeme *et al.*, 2009). This clearly indicated a high BTB prevalence in cattle at the livestock/wildlife interface as compared to an area outside the interface, such as Kuzungula (Munyeme *et al.*, 2009).

In cattle herds under the rural livestock production system in the Torodi region of Niger, the prevalence of BTB was found to be 3.6 % when tested using the comparative intra-dermal skin test (Boukary *et al.*, 2011). Still in the Niger, in carcasses tested by a detailed *post mortem* in an abattoir in Niamey, gross lesions typical of *M. bovis* infection were found in 0.19 % of the cattle, 0.11 % of the camels, 0.001 % of the sheep and 0.006 % of the goats (Boukary *et al.*, 2012).

The Mejia canton region in the North of Ecuador is a major dairy cattle producing area and the prevalence of BTB in both the dairy animals and the dairy herds was tested on two consecutive years in 2007 and 2008 using the comparative intra-dermal skin test. The BTB prevalence in the dairy animals was 7.41 % and 7.13 % while the herd prevalence was found to be 55 % and 65 % respectively in the consecutive years (Proano-Perez *et al.*, 2006). In a preliminary study in the same region, where BTB prevalence was studied using both the single intra-dermal skin test and the comparative intra-dermal skin test, the BTB prevalence had been found to be 4.24 % and 3.85 % for the two test respectively (Proano-Perez *et al.*, 2006).

In Northern Tanzania, Cleaveland *et al.* (2007), found a BTB prevalence of 0.9 % and a herd prevalence of 11.8 % while Gumi *et al.* (2011) found a BTB prevalence of 5.5 % and 7.0 % when using a cut-off of > 4 mm and > 2 mm respectively for a test positive animal, in the Oromi region in Southern Ethiopia. The latter also reported a herd prevalence of 41.9 % and 48.4 % for the two respective cut-off points (Gumi *et al.*, 2011).

BTB prevalence can be estimated using *post mortem* examinations at slaughter, based on lesions typical of *M. bovis* during routine slaughter and/or necropsy specific for BTB diagnosis, although the prevalence is calculated based on the number of carcasses found with lesions over the total slaughter over a fixed period of time (Demelash *et al.*, 2009). Using this method in five abattoirs in Ethiopia over a period of seven months between July 2006 and January 2007, Demelash *et al.* (2009), found a BTB prevalence of 10.2 %, with 20.5 % having generalized BTB lesions and the rest, being 79.5 % having localized lesions (Demelash *et al.*, 2009).

Inangolet *et al.* (2008) found a BTB prevalence of 1.3 % in cattle in the transhumant and agro-pastoral cattle herds in the border areas of Katakwi and Moroto districts in Uganda, using a comparative intra-dermal skin test. In the sub regions studied, the BTB prevalence ranged from 0 % in Ongogonja, Mogoro and Katakwi to 6.0 % in Kapujan (Inangolet *et al.*, 2008).

In a goat farm in Ireland, 66.3 % of the animals were found to be BTB positive when tested using the single intra-dermal tuberculin test during an outbreak of the disease, whose origin was traced to a recently introduced consignment of goats (Shanahan *et al.*, 2011). The outbreak was eventually controlled and the disease eradicated from the farm using the test and slaughter method, with the single intra-dermal test, the IFN- $\gamma$  and a multiplex immunoassay being used to identify test-positive animals (Shanahan *et al.*, 2011).

While assessing the prevalence of *M. bovis* infection in cattle and wild ruminants in a wildlife-livestock interface area of the highlands of Mexico, Cisneros *et al.* (2012), did not find any *M. bovis* in the wild ruminants, although inflammatory lesions were found in the lungs and lymph nodes, but the BTB prevalence in cattle was found to be 0.86 %.

Awah-Ndukum *et al.* (2012) used sero-prevalence and population-based tuberculin skin test surveys in the highlands of Cameroon, in 2009 and 2010, to estimate the prevalence of BTB in cattle, using comparative intra-dermal tuberculin skin test at three cut-off points for the test-positive animals, and a single intra-dermal skin test. The BTB prevalence in cattle was found to be 3.59 % - 7.48 %, 8.92 % - 13.25 %, 11.77 % - 17.26 % and 13.14 % - 18.35 % at the > 4 mm, > 3 mm, > 2 mm cut-off points for test positive animals and for the single intra-dermal skin test respectively, with the BTB prevalence characteristically increasing with the lowering of the cut-off point for test positive animals (Awah-Ndukum *et al.*, 2012).

Following a study on BTB prevalence in cattle, camel and goats in four pastoral associations in the Somali region in South East Ethiopia, Gumi *et al.* (2012), found that there was no significant difference between livestock species in positivity and concluded that the BTB prevalence was low in Somali pastoral livestock in general and in camels and goat in particular. This was after finding a BTB prevalence of 2.0 % in cattle, 0.4 % in camels and 0.2 % in goats, when using the comparative intra-dermal skin test.

In pigs, Arega *et al.* (2013), found a BTB prevalence of 5.8 % in Central Ethiopia, finding that the age and origin of the pig was significantly associated with the prevalence of BTB while no such association was found with the sex, the floor type and the water sources. *M. tuberculosis* was isolated from five of the twelve pigs with tuberculous-like lesions, suggesting a possible link of transmission of the pathogen from pigs to humans (Arega *et al.*, 2013). Kassa *et al.* (2012) also isolated *M. tuberculosis* from goats during a cross sectional study of BTB in 2231 small ruminants in the Afor pastoral region of Ethiopia, where the BTB prevalence was found to be 0.5 % and 3.8 % at the  $\geq 4$  mm and  $\geq 2$  mm cut-off for BTB positive ruminants respectively and a herd prevalence of 20 % and 47 % at the respective cut-off points.

Berg *et al.* (2008) also isolated *M. tuberculosis* from eight cattle that had tuberculous lesions during a BTB prevalence study in Ethiopia which was based on studying BTB cases identified due to the presence of tuberculous lesions and subsequent culture. Out of 32 800 cattle inspected, the researchers found tuberculous lesions in 4.7 % or 1 541 of them, although *M. bovis* was only isolated in 58 cases out of the 169 animals that showed positive for acid-fast

organisms (Berg *et al.*, 2008).

In a BTB cross-sectional study in Asmara, Eritrea, out of 1 813 animals from 72 herds tested for BTB using the comparative intra-dermal skin test, 14.5 % of the animals were positive reactors and 41.7 % of the herds had at least one positive reactor.

The importance of culling and/or segregating positive reactors was highlighted in two dairy farms in Northern India, where one farm had a relatively high BTB prevalence of 15.75 % in its Holstein-Friesian cows and their cross bred progeny while the other farm had a relatively low BTB prevalence of 1.85 % (Mukherjee, 2006). The former farm had never culled or segregated its positive reactors ever since the first animal tested positive in 1992, while the latter farm regularly tested, culled and/or segregated its positive reactors (Mukherjee, 2006).

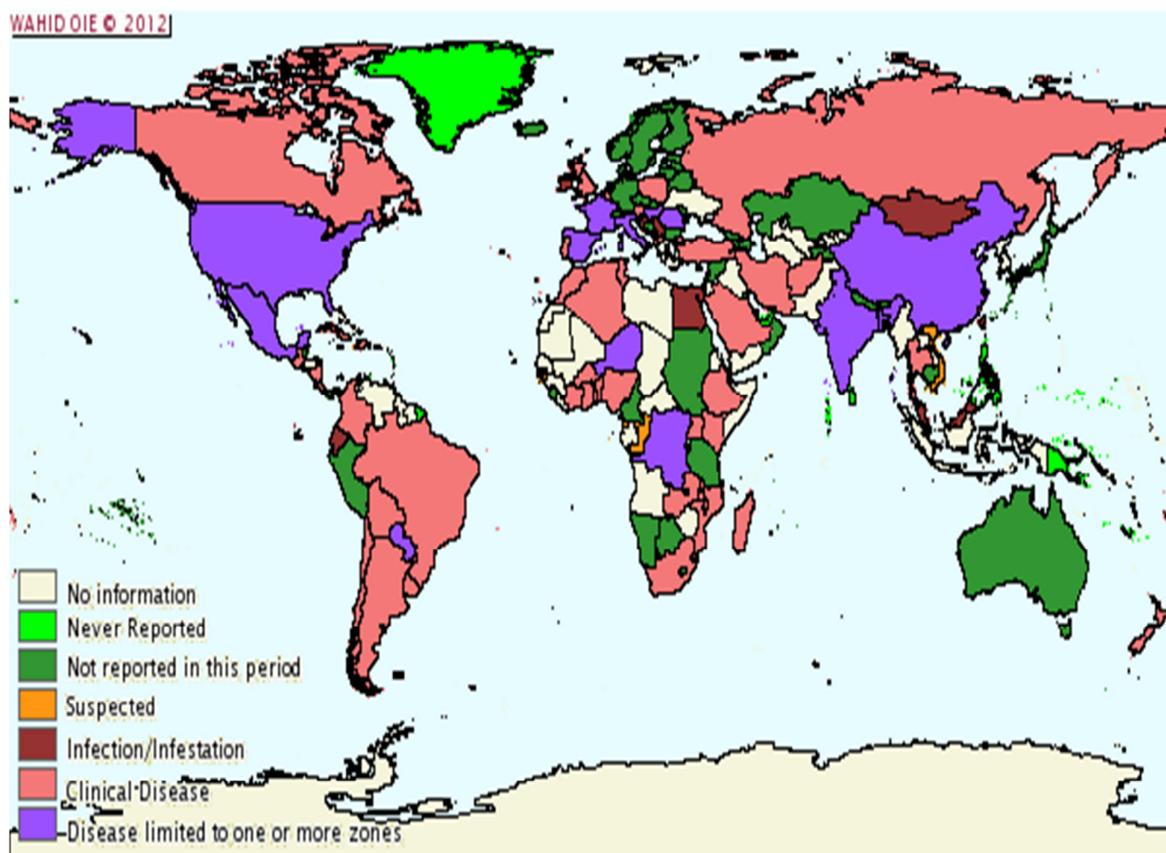
In the Dangme West district of Ghana, Bonso *et al.* (2000), found a BTB prevalence of 13.8 % in cattle, although the BTB prevalence was as high as 50 % in some kraals and it was twice as high in cows compared to heifers and bulls. In the sub districts of Dangme West, the BTB prevalence was 19.0 % in Ningo, 14.0 % in Dodowa, 11.3 in Prampram and 10.8 % in Osudoku (Bonso *et al.*, 2000).

Several studies have been performed to compare the prevalence of BTB in intensive and extensive management systems, with most of them indicating a higher prevalence of the disease in the former than in the latter. Shirima *et al.* (2003) compared the prevalence of BTB in pastoral and intensive production systems in the Eastern zone of Tanzania, using the single intra-dermal tuberculin skin test. The researchers found a significant difference on the BTB prevalence in the two systems, reporting a prevalence of 2.0 % and 1.0 % for the intensive and pastoral production system respectively.

These findings were collaborated by those found by Fetene *et al.* (2009) in three districts of North western Ethiopia, also using the single intra-dermal tuberculin skin test, finding a significantly higher BTB prevalence, at 22.1 %, in cattle under intensive production compared to the prevalence of BTB in cattle under extensive systems, which was 8.2 % (Fetene *et al.*, 2009).

In smallholder dairy, representing an intensive production system, and traditionally managed herds, representing an extensive production system, in the Tanga region of North-Eastern Tanzania, Swai *et al.* (2012), found no BTB in the latter system while they reported a prevalence of 2.0 % and a herd prevalence of 5.7 % in the former production system.

Although actual prevalence figures in the different management systems are not stated in their publications, Inangolet *et al.* (2008), claim that their study demonstrated a relatively low BTB prevalence of BTB in Zebu cattle reared under traditional husbandry systems in Uganda, suggesting a low infectiousness of the disease under such mode of production.



**Figure 1** A map of the world showing the incidence of bovine tuberculosis as reported to the OIE in the period January to June 2012

**Table 1** BTB prevalence in other studies (Tschopp *et al.*, 2009)

Country	Researcher	Year of Study	Bovine Bias	Prevalence
Tanzania	Shirima <i>et al.</i>	2003	Not stated	1.3 %
Tanzania	Cleaveland <i>et al.</i>	2007	Not stated	1.9 %
Ethiopia	Ameni <i>et al.</i>	2003	> 4 mm	7.9 %
Ethiopia	Ameni <i>et al.</i>	2007	> 4 mm	11.6 %
Ethiopia	Tschopp <i>et al.</i>	2009	> 2 mm	3.1 %
Zambia (Kafue Basin)	Munyeme <i>et al.</i>	2008	> 4 mm	30 %
Ghana (Greater Accra)	Bonsoa <i>et al.</i>	2000	Not stated	13.8 %
Nigeria (Bauchi State)	Joseph M	2012	Not stated	11.73 %
Chad (Southern Chad)	Bongo <i>et al.</i>	2009	> 2 mm	7.7 %

### Economic importance

BTB is a disease listed in the World Organisation for Animal Health (OIE)'s Terrestrial Animal Health Code, 2011, (Article 1.2.3) and must be reported to the OIE (Chapter 1.1.2, Notification of Diseases and Epidemiological Information). Economically, BTB causes production losses, especially in livestock, due to reduced milk yields, losses due to condemned carcasses or carcass parts at slaughter, and reduced fertility in affected animal populations (Michel, 2010). The high costs of controlling and eradication of the disease also has significant economic implications for the affected farmers and the state (Michel, 2010).

BTB causes a decrease in milk and meat production in cattle populations. However, Ameni *et al.* (2007), found that cows from negative and positive herds had the same physical conditions and milk production levels, even though the latter had sporadic deaths and breeding problems as cows failed to come to estrous and to breed (Ameni *et al.*, 2007). This is in contrast with a finding by Boland *et al.* (2010) who studied the effect of milk production in infected dairy herds in Ireland, taking into consideration both clinical and sub-clinical BTB infection. The researchers observed that milk yield was significantly lower for BTB reactor cows, with differences ranging from 120 kg in 2003 when the animals were in their third lactation to 573 kg in 2001 when the animals were in their first lactation, when compared to the non-reactor cows (Boland *et al.*, 2010). Confounding to this is the fact that BTB usually becomes

widespread in the population before being detected and hence increases its effect on the population and the cost of controlling it. It has high economic relevance within the context of livestock farming as it directly affects animal productivity and influences international trade of animal products (Munyeme *et al.*, 2012). In New Zealand, BTB has been identified as a major problem to agriculture as it poses a risk to export-market access (Barlow *et al.*, 1997).

In abattoirs, BTB causes partial or total condemnation of affected organs and/or carcasses respectively, causing a financial loss to farmers. BTB is responsible for the total and partial condemnation of a significant amount of all meat routinely inspected in abattoirs. Over twenty years ago, in 1992, Egypt condemned meat with a value of US \$ 5 million, a lot of money today, but at that time it was worth a fortune, while in the Ivory Coast, in the same year, 50 % of all condemned carcasses were due to BTB while 46 % of all partial carcass condemnation were attributed to BTB contamination (Benkirane, 1993).

BTB affects trade in livestock and livestock products, as livestock, and game such as the African buffalo, cannot be traded unless they are certified free of the disease. Eradication of BTB constitutes one of the essential bases for the establishment of the trade of animals and their products in the European Community, as well as the enhancement of the livestock productivity and the human alimentary safety (Lopez-Soria *et al.*, 2009).

On the other hand, abattoirs with full *ante* and *post mortem* inspection play a significant role in the control of BTB as the development process of the eradication programs usually shows a tendency to establish epidemiologic surveillance systems in abattoirs, where not only the sanitary inspection of all the animals slaughtered for consumption purposes is carried out but also the systemic collection of granulomas or lesions compatible with BTB for their subsequent culture, disease confirmation and epidemiological trace back of the outbreak (Parra *et al.*, 2007).

The existence of BTB in free-ranging mammals in Southern Africa poses significant threats to conservation and tourism (Renwick *et al.*, 2007). Southern Africa has many conservation areas which are popular tourist destinations, such as the Kruger National Park, racking in millions of dollars on an annual basis as revenue. In these tourist destinations, the so-called Big Five, the elephant, the buffalo, the lion, the leopard and the rhino, are used as a draw cards for tourists and with a BTB epidemic affecting a majority of them, tourism and conservation will be placed under pressure.

Game ranching is an important industry which is threatened by BTB, as it is known to affect wildlife species such as sable, antelope, bontebok and springbok, which play a significant role in game ranching (Michel, 2010).

Besides conservation, game ranching and tourism, BTB may pose serious threats to endangered species of wildlife living in the vicinity of free-ranging or captive infected wildlife (Renwick *et al.*, 2007). Unless treated, BTB infected animals eventually succumb to death and, with treatment of endangered wildlife difficult in the wild, BTB infection of endangered species may accelerate their extinction.

BTB may affect animals in zoological collections which are of high value, such as the chimpanzee, Malayan Tapir, Beaver, warthog, lechwe, waterbuck and the Capuchin monkey, threatening the very existence of zoological collections (Michel, 2010).

BTB may affect companion animals which are of economic and sentimental value to their owners, such as the marmoset, dog, horse, baboon and the parrot, as well as affecting animals which may be kept in rehabilitation centres, such as the velvet monkey (Michel, 2010).

Further economic implications of BTB are felt on the control and eradication of the human disease that it may cause (Michel, 2010). In the pre-pasteurisation age, *M. bovis* contributed significantly to human tuberculosis, mainly the extra-pulmonary form. The control of the 3.1 % portion of all human TB cases comes at huge costs to governments and the affected individuals.

As the above section (BTB in Selected Countries), has shown, there are documented cases whereby *M. tuberculosis* has been isolated from animals, indicating a spillover of human TB to dairy cattle and other animals, as in Ethiopia, South Africa, Nigeria, China and India (Michel, 2010). This further cements the zoonotic importance of tuberculosis as caused by Mycobacteria from the MTB complex, including *M. bovis*.

In conclusion, BTB affects livestock health causing production losses which has an economic effect besides it being a threat to the conservation of wildlife. In as far as humans are concerned; BTB is a threat to their health and has socio-economic effects to them.

## Host susceptibility

While cattle are susceptible to BTB, they also serve as reservoir hosts for the disease. Cattle can maintain a BTB infection within the population without an external source of infection from another animal species. Effective control of the disease in wild animal populations, such as the African buffaloes, has not been very successful. Some wildlife species, such as cheetah, leopards and impala, are dead end hosts or spillover hosts, meaning a BTB infection would generally die out in the population unless there is a continuous external source of infection. The behavior of such animals has been attributed to this fact, especially them being solitary animals, with most affected animals either recovering or dying without having had the opportunity to transmit the infection to others. BTB infection is easily maintained in wildlife populations where animals live in herds or larger groups, such as the African buffalo (*Syncerus caffer*) and greater kudu. In this case, an infected animal has ample opportunity to pass the infection to fellow herd members before it is eventually overcome by the illness.

Species like the greater kudu (*Tragelaphus streptoceros*) appear to maintain, spread and even drive a BTB epidemic, giving several dynamic options for the wildlife-wildlife transmission of *M. bovis*, such as buffalo to buffalo, buffalo to other unidentified wild life species such as the greater kudu- buffalo, and so on (Garine-Wichatitsky *et al.*, 2010).

Once BTB is established in a native free-ranging maintenance hosts, such as the buffalo and the greater kudu, eradication is unlikely (Garine-Wichatitsky *et al.*, 2010; Michel, 2012). Furthermore, control strategies in wildlife populations are limited but chances of success are greater if control measures are initiated at the earliest stage of disease spread into a new area (Garine-Wichatitsky *et al.*, 2010).

Most mammalian species are susceptible to BTB and the number of wild African mammal species in which BTB is being reported is increasing (Renwick *et al.*, 2007). This is due to the fact that increases in domestic livestock numbers and an expanding interface with wildlife has increased BTB infection pressure, a heightened passive surveillance and increased amounts of specific research due to public awareness of BTB and its economic and environmental impact, and active surveillance and monitoring in conjunction with improved *ante-mortem* diagnostics providing the means to assess the prevalence and incidence of BTB in wildlife (Michel, 2012).

## Zoonotic importance

Many countries in the Southern African region are faced with the serious challenge of HIV/AIDS in human populations. Tuberculosis has been identified as the major and potential fatal opportunistic infection in patients with HIV/AIDS infections leading to a serious co-infection status that accelerates the pathogenesis of both (Munyeme *et al.*, 2008). Due to the fact that the HIV pandemic has left sufferers immuno-compromised, cattle may be a significant source of zoonotic BTB infection for man (Munyeme *et al.*, 2008). *M. bovis* was isolated in two out of five patients with pulmonary TB in Zambia (Munyeme *et al.*, 2008). The HIV/AIDS pandemic has caused a dramatic increase in the number of open human TB cases and man is increasingly becoming a risk to cattle, as in rare cases, humans have infected cattle via aerosols or urine.

Person to person transmission is rare in immune-competent individuals but *M. bovis* has occasionally been transmitted within small clusters of people, particularly alcoholics or HIV infected individuals (Munyeme *et al.*, 2008).

While there are about nine million new cases of human tuberculosis and two million deaths that are reported worldwide each year, *M. bovis* globally accounts for 3.1 % of all human tuberculosis cases, which are broken down to 2.1 % of all pulmonary TB cases and 9.4 % of all extra-pulmonary cases (Tschopp *et al.*, 2009). This relates to 279 000 new human tuberculosis cases and 62 000 deaths reported on an annual basis. Of these astronomical figures, Sub-Saharan Africa displays the highest annual rate of infection with human tuberculosis, probably catalysed by HIV/AIDS pandemic (Tschopp *et al.*, 2009).

In Europe, there are 60 new cases of BTB in humans that are reported per year while in Latin America, 7 000 new cases of BTB in humans are reported annually (Michel, 2010). In Mexico, 13.8 % of all human TB cases are due to *M. bovis* infection (Michel, 2010). In Tanzania, *M. bovis* was isolated from 18-30 % of all samples taken from TB patients while it was isolated from 1 % of sputum samples from TB patients in Ethiopia (Michel, 2010). In Uganda and Nigeria, *M. bovis* infection in human TB patients was 6.9 % and 5.0 % respectively (Michel, 2010).

An earlier study that had analysed Mycobacterium strains originating from human sputum in Egypt had found that nearly 5 % of them were *M. bovis* although this relatively high percentage was attributable to the fact that the study population lived near the Cairo abattoir and some of them were abattoir workers (Benkirane, 1995).

Zoonotic transmission of BTB is negligible in most of the developed world while it is regarded as a neglected zoonosis in developing countries ([www.who.int/entity/zoonoses](http://www.who.int/entity/zoonoses)). Developing countries have a high number of vulnerable communities where diseases such as BTB which are transmissible between human beings and livestock impact directly on human health and threatening human livelihoods by compromising sustainable food supply, income and social status ([www.who.int/entity/zoonoses](http://www.who.int/entity/zoonoses)).

To obtain the best value for their money, beef farmers in Swaziland are encouraged to sell their livestock in official markets, both local and foreign. This usually requires that cattle are slaughtered in official abattoirs with full *ante-mortem* and *post mortem* inspection. As BTB has a long subclinical phase, farmers send healthy looking animals into abattoirs for slaughter, yet such animals could be already infected with subclinical BTB. Such cases are usually diagnosed at *post mortem* inspection. After confirmation by the laboratory, the entire affected carcass is condemned and the farmer does not receive any compensation for his/her animals. Such financial loss to the farmer, at the very end of his production stage, is difficult to accept from the farmers' perspective. For the import and export of bovines, and for some wild life like the African buffalo, BTB is one of the diseases requiring testing before a consignment of cattle or African buffalo is given veterinary clearance. Usually, this is done after the sales agreement has been made and some financial commitment carried out. The whole transaction is cancelled in the event of the cattle or African buffalo testing positive for BTB, leading to financial losses, especially for the farmer right at the end of the production cycle.

### **Effects of BTB on livestock farming**

Consequently, *M. bovis* infection has a significant economic impact with worldwide annual losses to agriculture estimated at US \$ 3 billion (Weller *et al.*, 2009).

Between 2003 and 2011, the Swaziland Meat Industries, the only abattoir in the country with an active BTB surveillance program, condemned carcasses worth US \$ 1,300,000.00 due to suspected BTB infection (Annual Report, 2012). The other abattoirs in the country, which are relatively smaller in terms of throughput or the number of cattle that are slaughtered only, condemn carcasses only if they are gross visible lesions typical of BTB over diffusely distributed on the carcass. Farmers who intend to import or export cattle are required by law to test them for BTB and only animals certified as free from BTB can be imported or exported.

### 1.1.2 Aetiology

Tuberculosis in cattle is caused by bacteria belonging to the *Mycobacterium tuberculosis* complex (MTBC). BTB is specifically caused by *Mycobacterium bovis* (Cousins, 2001).

The *M. tuberculosis* complex is considered to be a family of ecotypes of very closely related Mycobacteria, with each ecotype being adapted to cause tuberculosis disease in a specific host species/group but interspecies transmission do occur (Michel *et al.*, 2009).

*Mycobacterium caprae* causes tuberculosis in goats but *M. bovis* has been known to cause tuberculosis in goats although the importance of the disease caused by the latter varies depending on the country and the type of enterprise (Cousins, 2001). In much rarer circumstances, a transient mostly self-limiting form of tuberculosis in cattle maybe caused by the human pathogen, *Mycobacterium tuberculosis*.

*Mycobacterium species* are Gram positive rods with a diameter of about 0.3-0.6 micrometers and a length of 1.5-3 micrometers, which are acid fast (resistant to acid decolourisation agents) and non-spore forming. The rods may be straight or curved. The tubercle bacilli grow aerobically on *in vitro* cultivation at an optimal temperature of 37 °C. *M. bovis* grows very slowly as it only replicates every 12-20 hours (Wadhwa *et al.*, 2006). On culture, *M. bovis* colonies appear after about three to six weeks, whereby the organism is micro-aerophilic. However, isolates may only be observed at the end of 16 weeks hence a culture cannot be labeled negative for BTB until after it has been incubated for up to three to four months (Wadhwa *et al.*, 2006).

*M. bovis* can survive for several months in the environment, particularly in cold, dark and moist conditions, with the survival time ranging from 18-332 days in ambient temperatures of 12-24 °C, depending on the exposure to sunlight (Wadhwa *et al.*, 2006).

### 1.1.3 Epidemiology

#### Habitat

In the environment, *M. bovis* prefers cool and moist habitats (Blood *et al.*, 1992). It is resistant to most disinfectants but may be inactivated by UV light and direct sunlight. Hall *et al.* (2007) claim that hydrogen peroxide vapor is effective in decontaminating laboratory surfaces contaminated with *Mycobacterium tuberculosis*, as an alternative to traditional decontamination

methods. It is not clear whether the same can be said for *Mycobacterium bovis*. *M. bovis* can survive in frozen tissue (Corner, 1994).

### **Distribution and Host Range**

Most mammals, including wildlife and humans, are susceptible to one species or another of *Mycobacterium*. Most countries of the world are affected by BTB, including the whole of the SADC region. In developed countries, the disease is less common in cattle populations but more common in wild animal populations. This is due to the extensive control programs, usually involving eradication of infected cattle, which have been very effective in controlling the disease in cattle population. However, in some of these countries, the disease spilled over from cattle populations into wildlife, before eradication from cattle. Controlling the disease in wild animals is not as feasible as it is in cattle. The intra-dermal skin test, which has been extensively and successfully used in detecting infected cattle, is not very practical for detecting infected free ranging wild animals as it requires the physical handling of animals twice within 72 hour.

In developing countries, the disease is prevalent in cattle and wild animal populations, as well as other domestic livestock. The contrast to the developed world scenario is due to the inadequate or absent control measures in the cattle populations of African states.

In Swaziland, the estimated prevalence is less than 0.5 % and no cases have been reported in wild ruminants (Blood *et al.*, 1992). It is not clear whether the estimated prevalence and the lack of cases in wild ruminants are a reflection of the true picture or a result of the lack of active surveillance programs of the disease in non-domesticated species (Blood *et al.*, 1992).

As has been mentioned above, cattle and African buffalo are known to be the maintenance hosts for bovine tuberculosis. This is especially the case in African populations while in other parts of the world, other wildlife species are known to be the maintenance host for the disease: In New Zealand, the brush tail possum (*Trichosurus vulpecula*) and farmed fallow deer (*Cervus elephus*) (Barlow *et al.*, 1997). In England and Ireland, the badger (*Meles meles*) (Shanahan *et al.*, 2011); the deer (*Cervus elaphus*) and the wild boar in Europe (Lopez-Soria *et al.*, 2009), the bison (*Bison bison*) in Canada and the white-tailed deer (*Odocoileus virginianus*) in some parts of the United States of America (Thoen *et al.*, 2009). The African buffalo (*Syncerus caffer*) is also known to have exported the disease from cattle populations into other wildlife populations with its close proximity to livestock populations in some farming enterprises (Renwick *et al.*, 2007). The wild boar is an important maintenance and spillover

hosts in Spain and Portugal (Lopez-Soria *et al.*, 2009). Wildlife species which have been seen to be susceptible to BTB include, amongst others, the greater kudu (*Tragelaphus strepsiceros*), lion (*Panthera leo*), cheetah (*Acinonyx jubatus*), baboon (*Papio ursinus*), duiker (*Sylvicapra grimmia*), hyena (*Hyaenidae*), leopard (*Panthera pardus*), lechwe (*Kobus leche kafuensis*), impala (*Aepyceros melampus*), bush buck (*Tragelaphus scriptus*), giraffe (*Giraffa camelopardalis*), springbok (*Antidorcas maurus*), nyala (*Tragelaphus angasi*), and the hare (*Lepus europaeus*) (Thoen *et al.*, 2009).

There are other wildlife species that have been reported to have been infected by *M. bovis* and have a potential to be reservoirs for *M. bovis* such as the llama (*Lama glama*), fallow deer (*Dama dama*) and roe deer (*Capreolus capreolus*) (Thoen *et al.*, 2009).

Species like the greater kudu (*Tragelaphus strepsiceros*) appear to maintain, spread and even drive a BTB epidemic, giving several dynamic options for the wildlife-wildlife transmission of *M. bovis*, such as buffalo- buffalo, buffalo- unidentified wild life species such as the greater kudu- buffalo, and so on (Gariné-Wichatitsky *et al.*, 2010).

In Southern Africa, the African buffalo (*Syncerus caffer*) and the Kafue lechwe (*Kobus leche*) have been identified as the major maintenance hosts for BTB but the role and importance of other host species as maintenance hosts and in spreading the disease is becoming apparent, even though it varies widely depending on a number of factors (Renwick *et al.*, 2007). These factors includes the spatial distribution and resource utilization patterns of the species, disease susceptibility and transmission modes in that species as well as the ecology of both the host(s) and vector(s) (Renwick *et al.*, 2007). These host species, which can all act as maintenance hosts for BTB, include the brush tail possum, European badger, bison and the white-tailed deer, and they allow for persistent infection in the wildlife population, enabling horizontal transmission of the pathogen between species (Renwick *et al.*, 2007).

The above host species contrast sharply with host species such as the lion, the leopard, cheetahs and other carnivorous hosts which cannot maintain infection in the absence of an infected maintenance host in the system (Renwick *et al.*, 2007). Due to these' host species limited ability to maintain the disease in the population on their own, they are referred to as dead-end or spillover hosts (Renwick *et al.*, 2007).

Swaziland boasts a diverse wildlife population, with most species of wildlife being represented in varying numbers, naturally occurring in the wild, in nature reserves and game parks, game farms and ranches ([www.swaziparks.com](http://www.swaziparks.com)). The largest nature reserves are owned by two conservation groups, namely the privately owned Big Game Parks that own Mlilwane, Hlane and Mkhaya nature reserves; and the state owned Swaziland National Trust Commission that own the flagship Malolotja Nature reserves and a few others such Mlawula and Mantenga ([www.swaziparks.com](http://www.swaziparks.com)). The biggest game ranch in the country is called IYSIS and is privately owned ([www.kingdomofswaziland.com](http://www.kingdomofswaziland.com)).

Wildlife plays significant economic and socio-conservatory roles in the country in as far as conservation, cultural activities such as the annual royal hunting party, ecotourism including trophy hunting and other consumptive conservation practices such as the sale of game meat following culling([www.kingdomofswaziland.com](http://www.kingdomofswaziland.com)).

However, as in other countries, wildlife in Swaziland has the potential of being a chronic source of BTB infection for cattle and thereby maintaining BTB in cattle in the presence of control programs. Once infected, many wild animals have shown the potential to act as reservoirs of infection for both domestic cattle and other wildlife species (Renwick *et al.*, 2007).

## **Transmission**

The transmission of BTB is complex and there is scientific evidence that supports both cattle-cattle and wildlife to cattle transmission routes (Skuce, 2012). Infected animals represent the principal source of infection and the transmission of *M. bovis* from infected animals to susceptible wild life, and *vice versa*, are highest when they share pastures and territory (Skuce, 2012). Infected animals excrete the organisms in exhaled air, in sputum, in faeces, milk, urine, vaginal, uterine discharges as well as discharges from open peripheral lymph nodes, saliva and discharging lesions (Tschopp *et al.*, 2009). Faeces, urine and milk are thought to be minor sources of transmission when compared to the others (Wadhwa *et al.*, 2006). The excretion of *M. bovis* in nasal mucus is a consistent feature in all infected cattle and shedding rates ranging from 6 % - 20 % are found amongst naturally infected tuberculous cattle in different countries but the shedding of *M. bovis* in nasal and oral discharges is an infrequent event in the African buffalo, although it may occur in limited amounts in buffalo with clinical signs or in such low quantities as may not be detected by routine testing (Michel *et al.*, 2007). The primary route of transmission becomes the exchange of respiratory secretions between infected and non-infected animals primarily through the inhalation of aerosol droplets that have been exhaled by an infected animal (Wadhwa *et al.*, 2006). This is the respiratory route where Mycobacteria

from open pulmonary lesions are aerosolized in the respiratory tract (Renwick *et al.*, 2007). This classical mode of transmission is the main cause of disease spread within human and cattle population especially, in human populations, where certain traditional societies in Africa that have close contact with their cattle, often sharing living space with them, providing an ideal situation for people to inhale aerosolized *M. bovis* and for infected people to infect their livestock (Renwick *et al.*, 2007).

The alimentary tract infection may occur through the excretion of Mycobacteria in sputum, draining sinuses, faeces or urine of an infected and the subsequent consumption of contaminated materials by other susceptible animals (Renwick *et al.*, 2007). Susceptible animals may be infected through ingesting feed, on the pasture/veld or in troughs as well as drinking water that may have been contaminated with the pathogenic organism excreted by an infected animal. Secretions and fecal material may contaminate feed, grazing pastures as well as communal drinking water. Faecal material remains infective for 6-8 weeks in slurry (Selman, 1981).

This type of alimentary tract infection is common in non-aggressive herbivorous species sharing range or habitat niches, with transmission occurring mainly through the consumption of contaminated vegetation (Renwick *et al.*, 2007).

A second alimentary tract infection may occur in aggressive inter-species encounters and prey to predator transmission, whereby transmission of the pathogen to the predator species occurs through the consumption of infected materials such as lesions, tissues, internal organs, etc., by a susceptible individual (Renwick *et al.*, 2007). This mode of transmission is a threat to carnivores living in areas with infected prey species and to rural pastoralists, small-scale farmers as well as general consumers in Africa that consume products from infected animals such as unpasteurized milk (Renwick *et al.*, 2007).

The percutaneous route is less known but is documented in the Kudu where contaminated thorns may scratch or abrade the delicate or facial skin of the species and in large predators where fight wounds contaminated by *M. bovis* result in chronic granulomatous infection of the skin, subcutis or muscle (Renwick *et al.*, 2007).

In wildlife populations, buffalo to buffalo contact may transmit *M. bovis* from infected buffalo herds to un-infected one due to animal movement in between herds as buffaloes, especially bulls and young heifers frequently move from herd to herd and may contribute to the spread of *M. bovis* by mixing with unexposed herds (Gariné-Wichatitsky *et al.*, 2010).

The transmission of *M. bovis* to young animals, such as calves, through milk has been supported by the isolation of *M. bovis* in milk and faeces from milking buffalo and cattle in Nepal, and the high prevalence of *M. bovis* in the mammary glands and young tuberculin positive animals in abattoir surveillance in Chad (Tschopp *et al.*, 2009).

Amongst cattle, the transmissibility of BTB is relatively low (Neill *et al.*, 2001). This is indicated by the small number of tuberculin-reactors usually found in *M. bovis* infected herds (Neill *et al.*, 2001).

In infected cattle, BTB presents predominantly as a disease of the respiratory tract and hence the bacilli excreted in aerosolised form, especially from the lungs, represent the most widely recognized and efficient source of BTB infectivity (Neill *et al.*, 2001). Hence it is widely accepted that cattle are most likely to be infected with *M. bovis* through inhalation of aerosolised droplets containing viable bacteria (Rodgers *et al.*, 2007). The size and consistency of aerosolised droplets appear to be a crucial factor in BTB transmission (Neill *et al.*, 2001). Fine aerosols suspensions of low viscosity are more effective in delivering the mycobacterium pathogen relative to bigger aerosols of high viscosity (Neill *et al.*, 2001).

The disease spreads when an infected animal is introduced into a non-infected herd. Indirect *M. bovis* challenge models that place cattle in contact with infected cohorts, wildlife or a contaminated environment have provided information on transmission and pathogenesis of disease by reproducing pathological change typical to that observed in field cases (Rodgers *et al.*, 2007).

Farm management and cattle husbandry practices, such as silage storage, the spreading of slurry on pastures, the use of certain housing types and farms having multiple premises can influence the transmission of BTB (Reilly *et al.*, 2007). *M. bovis* infection in a herd can either be sporadic or short lived while in other herds it can be sporadic, and there are biological differences in the transmission dynamics in these different herds (Reilly *et al.*, 2007).

Barlow *et al.* (1997), using deterministic and stochastic versions of simulation models of BTB disease transmission, illustrated that within-herd BTB transmission does occur within cattle herds in New Zealand. The researchers further on defined a mass-action BTB disease co-efficient, which is the proportion of susceptible animals infected per unit time per infectious animal, whose value is about  $2.7 \times 10^{-5}$  per animal per day for a typical herd of about 200 animals (Barlow *et al.*, 1997). This means that the number of potentially infectious contacts made per infectious animal per day, referred to as the contact rate, is 0.0073 (Barlow *et al.*, 1997). The estimates of the mass-action BTB disease co-efficient and the contact rates are true for both beef and dairy animals and could be extended to other herds worldwide with similar parameters (Barlow *et al.*, 1997).

However, the disadvantage of using conventional simulation models for the transmission of BTB is that they are mainly based on the assumption that animals become detectable by testing before they become infectious, whereas an alternative scenario exist whereby there is no period of epidemiological latency before animals become infectious (Conlan *et al.*, 2012).

In infected farms, cattle-to-cattle transmission of *M. bovis* can be significantly reduced by the physical separation of reactors and non-reactors, having been identified by testing (Ameni *et al.*, 2007). Physical separation of infected and non-infected animals, after testing all animals in a herd, is an effective control strategy for BTB, although it is necessary to sustain the testing and segregation to ensure a low transmission of *M. bovis* in the herd (Ameni *et al.*, 2007).

### **Predisposing factors**

Several factors contribute to the spread of the disease within a herd. The number of infected animals within a herd increases the rate at which the disease will spread within the herd, as well as overcrowding and animals kept under shelter (housing) (Renwick *et al.*, 2007).

In Ethiopia, it was demonstrated that BTB prevalence rates were directly related to animal husbandry, with intensive farming practices showing high BTB prevalence rates compared to agro-pastoral and pastoral settings, which are non-intensive (Berg *et al.*, 2008). Furthermore, husbandry affects the intensity and distribution of pathology of BTB with the severity of the disease significantly greater in cattle kept indoors at a high density than those kept in pasture (Ameni *et al.*, 2006).

Housing cattle predisposes them to infection by *M. bovis*, as the closer the animals are packed together, the greater the chance that the disease, if present, will be transmitted (Ameni *et al.*, 2006).

Close contact between an infected animal and an uninfected animal is usually required for the spread of the disease (Renwick *et al.*, 2007). This is common in cattle farming enterprises where cattle are herded in groups, under intensive farming methods (Renwick *et al.*, 2007). Close contact is a predisposing factor to BTB as it facilitates the transmission of infective aerosols between animals (Cousins, 2001).

In intensive farming operations, where close contact, overcrowding and packed animals is a common feature, stress due to the uncomfortable physical conditions may contribute to the severity of the disease, although the stress may also be due to nutritional differences in housed and non-housed husbandry systems (Ameni *et al.*, 2006).

Lesions distributions in cattle kept in houses and/or intensive management systems differed significantly to that of cattle kept in pastures under a low intensive management system, suggesting different transmission routes of the BTB depending on the method of cattle husbandry (Cousins, 2001). Cattle kept in houses and/or under intensive management systems predominantly have lesions in the respiratory tract, indicating that inhalation could be the most likely route of infection, while cattle kept in pastures under low intensive management systems predominantly have lesions in the digestive tract, suggesting pathogen ingestion as the most likely route of infection (Cousins, 2001).

In extensive farming methods, the disease may spread due to certain farming practices, such as the use of communal kraals/pens, drinking points or dipping points. In Swaziland, all cattle which belong to a dip tank area gather at the dip tank every week in some regions or fortnightly in other regions for dipping purposes.

### **Routes of Infection**

The principal route of infection appears to be the oral-nasal route, with the inhalation of bacteria containing aerosols shed by infected animals, from coughing and sneezing animals, and the ingestion of contaminated feed, pasture and drinking water as the bacterium is shed in exhaled air, sputum, faecal material, milk, urine, vaginal and uterine discharges (Blood *et al.*, 1992). The

ingestion of contaminated products is considered to be a secondary and a less important route of transmission of *M. bovis* (Blood *et al.*, 1992). Certain management practices which are intensive in nature predisposes cattle to infection i.e. zero grazing and keeping livestock indoors (Renwick *et al.*, 2007).

Infected cattle excrete the bacilli in aerosols that contaminate the environment and hence other cattle grazing on the pasture or feeding from troughs contaminated with faecal material or urine also become infected (Rua-Domenech, 2006).

Other possible routes of transmission include congenital, cutaneous, use of infected semen, venereal and via the teat canal. In areas and/or farms with a high prevalence of bovine tuberculosis, a small number of calves are born with the disease. Calves drinking infected milk also get infected, with the organism entering through the gastro-intestinal tract (Blood *et al.*, 1992).

The Zebu breed of cattle is thought to be less affected by BTB than other breeds, unless when they are kept under feedlot conditions for longer periods (Blood *et al.*, 1992). The degree of infection in beef cattle maybe relatively lower than those in dairy due to the open range conditions which they are usually kept at and the excessive production stress associated with dairy animals (Blood *et al.*, 1992). The disease has a relatively low morbidity and mortality rate.

Fomites such as thermometers, cages, masks and containers used for feed and water are definite sources of infection in cases where severe shedders of the bacilli are involved (Thoen *et al.*, 2009).

### **Pathogenesis**

The incubation period of BTB may vary from a few weeks to months and even to years (Krishnappa *et al.*, 2006). This is due to the fact the development of a disease depends on the closeness of contact, extent of the disease, positivity of the source case (dose infection) and host parasite relationship (Krishnappa *et al.*, 2006).

Some infections of BTB may result in self-limiting lesions while others may develop into a systematic disease depending on a combination of factors, involving the physiological status of the host, in particular its immune status, age, and the infecting organism. The immunity status

of the animal, particularly cell mediated immunity and its protective immune response are crucial for the final outcome of infection. Cell mediated immunity is the major means of defense against tuberculosis (Widdison *et al.*, 2009).

## **Pathology**

Tuberculous granulomas may be found in any of the lymph nodes and other organs. Lymph nodes usually affected are the mediastinal and the bronchial lymph nodes. Ameni *et al.* (2007) reported a significant difference in the occurrence of BTB lesions in the lymph nodes, with the mesenteric lymph nodes being the most affected, followed by the retropharyngeal and caudal mediastinal lymph nodes respectively. Bronchopneumonia and/or miliary abscesses are found in the lungs. Pus is usually in the form of yellowish crumbly cheese (Blood *et al.*, 1992). Lesions are usually encapsulated. Abscesses and miliary lesions may be found in a number of body organs.

Lesions appear as firm nodules, white to yellowish in colour and are frequently of small sizes (Neill *et al.*, 2001). Macrophages are found within the infected foci with distinctive epithelioid cell appearance (Neill *et al.*, 2001). There are often observed as giant cells formed by macrophage fusion (Neill *et al.*, 2001). The epithelioid cells and giant cells form the centre of the developing tubercle and are later surrounded by a zone of lymphocytes, plasma cells and monocytes (Neill *et al.*, 2001). The tubercle develops a peripheral fibroplasias and central caseous necrosis within which mineralization can be observed (Neill *et al.*, 2001).

The *M. bovis* bacilli inside the tubercle can continue to multiply logarithmically if macrophages are not activated (Dannenberg, 2001). Hence the host is bound to destroy macrophages that contain bacilli. This is achieved by cytotoxic T cells and anoxia from damaged to their blood supply (Dannenberg, 2001). This form of immune response that culminates in the destruction of infected macrophages is referred to as tissue damaging delayed-type hypersensitivity (DTH).

DTH causes necrosis of tubercle foci and lung damage (Dannenberg, 2001).

In the lungs, lesions may either be unilateral or bilateral. Granuloma formation starts at the bronchi alveolar junction, extending into the alveolar and secondary lung lesions arising by hematogenous spread or intrapulmonary airways (Neill *et al.*, 2001). Alveolar macrophages may destroy *M. bovis* if inhaled in low doses otherwise they multiply intracellular within non-

activated monocyte/macrophages that enter the alveolus from the blood stream (Dannenber, 2001).

Pleural tuberculosis results either from direct expansion of sub pleural lesions or spread via the lymph or blood (Neil *et al.*, 2001). It is characterized by nodular lesions occurring in clusters and is heavily calcified.

Lung lesions are found only in a small proportion of tuberculous cattle especially in abattoirs where meat inspection and lung examination in a moving slaughter line is not very thorough and critical (Neill *et al.*, 2001). A majority of lesions are found in lymph tissue, especially the bronchial and mediastinal lymph nodes, head region lymph nodes such as the retropharyngeal and sub-maxillary; and in tonsils (Neill *et al.*, 2001). Ameni *et al.*, (2007) reported a significant difference in the severity of pathology of BTB in Holstein than in crosses and Zebu cattle (Ameni *et al.*, 2007).

## **Immunity**

The immune responses of the host to *M. bovis* infection are variable at the different severity stages of pathology of the disease (Ameni *et al.*, 2010). For an animal with a strong and intact immune system, i.e. with no factors that advocate immune-suppression or compromised immunity, the infection is usually self-limiting with minor lesions. If transmission is through inhalation, the alveolar macrophages destroy the pathogen, preventing the establishment of the disease. For animals with a compromised immunity, such as those which may be malnourished or already suffering from another infection, the infection may end up being a life threatening systemic disease.

Cell mediated Immune (CMI) response is the dominant response when an animal is infected with *M. bovis* and in most cases, there is little or no detectable antibodies (Neill *et al.*, 2001). CMI is a Th1 lymphocyte response to the antigens of the tubercle bacillus (Dannenber, 2001). The lymphocyte produce cytokines, such as interferon-gamma (IFN- $\gamma$ ) and expand the antigen-specific T-cell population. CMI is organ specific in its resistance to infection and, consequently, tubercle bacilli grows well in the lung where oxygen tension is high (Dannenber, 2001). However, other factors come into play in determining the success of CMI response to BTB infections.

When the bacilli enter the lung tissue or a local lymph node, they first multiply to cause a primary microscopic lesion, from which the infection can spread. Macrophages are stimulated and then they start engulfing the bacilli. The interplay between bacilli production and engulfing by the macrophages determines the fate of the infection. If the host's condition is such that its body is able to produce macrophages at a higher rate than bacilli production and the former are able to efficiently engulf the latter, then a self-limiting infection occurs.

However, if bacilli replication is at a rate higher than they are being engulfed by the macrophages, there is a numerical growth of the bacilli in the host. This activates a T-cell response in the host's body. The T-cell response involves the release of cytokines which mediate the phagocytosis of the bacilli. The infection is controlled if the T-cell response is strong enough and both the T-cells and the macrophages successfully engulf the bacilli. The infection persists if the T-cell response, together with the macrophages, is unable to arrest the infection.

It has been demonstrated that evidence of lymphocyte proliferation and interferon gamma assay responses coincided with immunopathogenic lesion development (Neill, 2001). With progressive disease, the interaction of the pathogen, *M. bovis*, and the macrophages precede immunopathological events involving predominantly cell-mediated immune responses. This results in distinct lesion formation (Neill *et al.*, 2001). With further lesion formation, the lesions become extensive and disseminated. At the same time, the animal becomes anergic, meaning unresponsive in CMI tests due to a switch from a TH1 to TH2 type of immune response (Neill *et al.*, 2001).

The immune status is particularly important in human beings. Many people who are suffering from HIV/AIDS have a compromised immunity and subsequent tuberculosis infections lead to a systematic disease.

The route of infection in cattle is significant to the pathogenesis of BTB. When the causative organism is inhaled or ingested, it is drained to the local lymph node and a lesion usually develops at the lymph nodes nearest to the point of entry, after spreading in macrophages via the lymphatic vessels into the local lymph nodes. The lesions are caused by multiplication of the infectious organism in the local lymph nodes. These initial lesions are referred to as the primary complex. The primary lesion develops by calcification and the necrotic focus is surrounded by granulation tissue and lymphocytes forming a tubercle or granuloma. A tubercle is an attempt by the host's immune system to arrest the infection (Dannenberg, 2001).

Granuloma formation within the lung and secondary lymphoid structures of the host is one of the defining features of BTB (Widdison *et al.*, 2009). Granulomas act to control the spread of infection but can also lead to extensive tissue damage and loss of function (Widdison *et al.*, 2009). They consist of multiple cell types, including infected and uninfected macrophages surrounded by B and T lymphocytes. The interaction of these cells leads to the mounting of an effective immune response (Widdison *et al.*, 2009).

Dissemination of the bacteria from the primary complex, in non-self-limiting infection, is variable in rate and route. It may be in the form of acute miliary tuberculosis, discrete nodular lesions in various organs or chronic organ tuberculosis. The infection may spread to affect a local area, as in the lung, or spread via the lymphatic vessels or blood to infect other organs and tissues. This process is called post-primary dissemination (Widdison *et al.*, 2005).

Body organs such as the lungs, bone, kidneys and meninges are referred to as vulnerable sites. In body organs that are not vulnerable sites, the infection is controlled by cell mediated immunity (CMI). In the vulnerable sites, CMI only reduces the infection but is not able to eliminate it (Cassidy, 2005).

The organism survives intracellularly in host cells by avoiding being phagocytised by the production of sulfotides and heat shock proteins. Subsequent macrophage and lymphocyte activity contribute to cell death and tissue destruction which causes caseous necrosis. Liquefaction and cavity formation may occur due to enzymatic actions on protein and lipids (Cassidy, 2005). The end result of all these attempts by the body to fight the infection is the formation of the granuloma. Bursting of a granuloma may lead to the spread of the infection while in other cases the granuloma may become encapsulated and be well organized by connective tissue (Cassidy, 2005).

In naturally infected cattle, tuberculosis lesions are found most frequently in the dorso-caudal, apex, region of the lungs, frequently close to the pleural surface but the size of inhaled infectious droplets and the topographic orientation of the lungs within the body plus the dictates of airflow dynamics determine lesion distribution (Cassidy, 2005).

Cassidy, 2005, claims that BTB controlled programs which are long standing have an influence on the distribution of lesions within an animal. He claims that disseminated disease or extensive pulmonary diseases are rare occurrences in countries with long standing eradication

programs; and, furthermore, lesions are found in other organs other than the lungs (Cassidy, 2005).

However, in some cases where cattle are positive for BTB in the intra-dermal skin test, there may be no visible lesions (NVL) during gross examination (Corner, 1994). NVL may be due to early infection, poor necropsy technique or infection with *Mycobacteria* other than *M. bovis* (Corner, 1994).

A host's type-1 immune response to BTB infection and non-pathogenic *Mycobacteria* suppresses the type-2 immune response to controlling helminth infection, consequently, a high prevalence of BTB infection may inhibit type-2 immune response resulting in high endoparasitic loads at the herd level in buffaloes (Garine-Wichatitsky *et al.*, 2010).

### **Human Disease**

Human tuberculosis is caused by *Mycobacterium tuberculosis*. However, humans are as susceptible to *Mycobacterium bovis* as they are to *M. tuberculosis* (Rua-Domenech, 2006). *M. bovis* also infects humans, causing zoonotic TB through ingestion of contaminated animal products such as raw milk, inhalation of bacteria containing dust particles and aerosols shed by infected animals and less frequently, by contact with mucous membranes and broken skin (Rua-Domenech, 2006). In humans *M. bovis* can be transmitted through the air from person to person, causing lung infection and could cause point epidemics in high risk population such as in HIV-infected people ([www.clubofmozambique.com](http://www.clubofmozambique.com)). The incidence of zoonotic TB associated with oral/gastrointestinal (milk or meat borne) respiratory (airborne) and cutaneous/mucosal transmission depends on the efficacy of BTB control programs in cattle, food hygiene measures and the cohort of the population under consideration (Rua-Domenech, 2006). The most common route of transmission is the consumption of raw milk of infected cows. In Swaziland, a majority of the population in the rural areas consume milk obtained from the subsistently farmed beef herd, exposing them to the risk of BTB infection if some of these cattle are infected. The zoonotic TB caused by *M. bovis* in humans is indistinguishable clinically or pathologically from TB caused by *M. tuberculosis* (Rua-Domenech, 2006). This maybe be due in part to the fact that strains of *Mycobacteria* share 99 % of their DNA sequence (Rua-Domenech, 2006). In order to differentiate the actual causative organism of human TB between *M. bovis* and *M. tuberculosis*, bacteriological cultures of clinical specimens have to be done, followed by typing of isolates according to growth characteristics, biochemical properties, routine resistance to pyrazinamide and specific non- commercial nucleic acid techniques (Rua-Domenech, 2006).

Rabbit inoculation was used to distinguish between *M. bovis* and *M. tuberculosis* in laboratory trials, by exploiting the fact that *M. bovis* is much more virulent than *M. tuberculosis* when inoculated in rabbits (Dannenbergh, 2001). However, this technique is no longer being used and has been replaced by PCR.

The pathogenesis in humans is also as described above, irrespective of whether the causative organism is *M. tuberculosis* or *M. bovis*. However, the pathogenesis in cattle is not as well understood as that in humans (Neill *et al.*, 2001). The processes involved in the disease in cattle have been drawn directly from knowledge of human infections modeled in ruminants (Neill *et al.*, 2001). These are thought to be accurate but they may not necessarily reflect precisely events in bovine tuberculosis.

In humans, *M. bovis* is mainly associated with extra-pulmonary tuberculosis, but depends largely on the route of transmission of the pathogen (Jain, 2011). Patients have largely compromised lung functions and young children have abdominal infections while older patients have swollen and/or ulcerated glands in the neck region ([www.clubofmozambique.com](http://www.clubofmozambique.com)). In Tanzania, in 2006, during a study in rural villages, 10.5 % of people with stomach or lymph gland tuberculosis were infected with *M. bovis* while the pathogen was also isolated from seven out of 65 patients with cervical adenitis, with only one of the patients coming from a household that owned infected cattle (Cleaveland *et al.*, 2007).

However, in recent years, the proportion of pulmonary cases in humans due to *M. bovis* appears to gradually increase (Jain, 2011). South Africa currently experiences one of the world's highest national incidence rates of all forms of TB (600/100 000) with an extremely high prevalence of culture-positive pulmonary TB (10/1000) (Parsons *et al.*, 2012). While animal to human transmission of *M. bovis* may occur, there is a significantly huge transmission of *M. tuberculosis* from human to human, making the animal-human transmission of *M. bovis* to be insignificant (Parsons *et al.*, 2012). However, transmission through unpasteurised cow's milk remains significant, especially on farms where gate sales of raw milk are allowed and, where pasteurisation is nonexistent, as in the case of Swaziland (Jain, 2011). Some approved establishment may also retail milk that has not been pasteurised. Occupational exposure to infectious aerosols from affected animals and their carcasses, especially abattoir workers, is also a possible mode of transmission (Jain, 2011).

Companion animals living in contact with TB patients are at great risk of exposure to the causative pathogens and hence there is a great potential for diseased companion animals to act as reservoirs of human infection (*M. tuberculosis* and *M. bovis*) (Parsons *et al.*, 2012).

Theoretically, consumption of raw and/or undercooked meat and meat products from tuberculosis animals could present a mechanism for human infection, although the evidence for human *M. bovis* infection attributable to ingestion of meat from tuberculous animals is very weak or non-existent (Munyeme *et al.*, 2008). The overall risk of human *M. bovis* infection arising from the consumption of meat from tuberculous cattle is low, especially in developed countries (Munyeme *et al.*, 2008). This may be partly due to the fact that BTB lesions in muscle are rare and only observed in animals with advanced infection. Secondly, especially in developed countries, bovine carcasses undergo *post mortem* inspection and the affected organs are condemned if the infection is localized or the entire carcass condemned if the infection is generalized. This tends to limit the exposure of humans from meat infected with BTB. However, in third world countries, a significant portion of the meat consumed does not undergo meat inspection and hence humans get exposed, increasing the overall risks of humans getting TB from the consumption of meat. As has been mentioned above, the HIV pandemic tends to worsen the issue. Rua-Domenech (2006), summarizes the salient point by stating that “the risk posed by eating undercooked meat of tuberculous animals maybe marginally greater in developing countries, where *M. bovis* infection in animals can be quite prevalent but veterinary controls (including meat inspection) are only sporadically applied”.

Human beings are infected with *M. bovis* principally from bovines and transmission from other animals other than cattle occurs only sporadically (Griffin *et al.*, 2006). Tuberculous deer can act as a potential source of *M. bovis* infection for farmers, veterinarians, abattoir workers, meat inspectors, animal handlers, hunters, game keepers and dealers (Jain, 2011). In New Zealand, trappers handling infected opossum have developed cases of TB.

There is a limited direct or indirect risk of transmission of *M. bovis* from companion animals to human beings (Parsons *et al.*, 2012). In environments with a high incidence rates of human TB in the population, there is a high level of transmission of *M. bovis* and *M. tuberculosis* between people and it can be expected that companion animals living in such environments will be of particular risk of infection by this pathogen (Parson *et al.*, 2012). In 2005, cases of human TB from presumptive feline TB and canine TB have been confirmed in Great Britain. Such is common when companion animals live in areas with a high incidence of TB in cattle and when the owners choose to ignore veterinary advice and illegally treat rather than euthanize

suspected infected companion animals (Rua-Domenech, 2006). Treatment of TB infected animals is not allowed except for expensive/valuable animals in zoos.

While BTB is fully curable in humans, the biggest challenge is in accurately diagnosing a human TB case caused by *M. bovis* ([www.clubofmozambique.com](http://www.clubofmozambique.com)). The treatment of *M. bovis* in humans slightly differs from *M. tuberculosis* as *M. bovis* is inherently resistant to pyrazinamide, the standard TB treatment drug. However, strong TB drugs such as isoniazid, rifampicin and ethambutol are still very active against *M. bovis* ([www.clubofmozambique.com](http://www.clubofmozambique.com)).

#### 1.1.4 Clinical Signs

##### General

Individual tuberculous cattle can differ markedly in clinical presentation and pathology (Neill *et al.*, 2001). The disease is usually a chronic debilitating disease but can occasionally be acute and rapidly progressive (Wadhwa *et al.*, 2006).

While *M. bovis* can infect a wide range of tissues in cattle, in developed countries with BTB control programs, the disease manifests as predominantly a respiratory infection (Rodgers *et al.*, 2007). In most field cases, the disease is observed in the lower respiratory tract, mainly the lungs and associated lymph nodes although the upper respiratory tract and associated tissues may also display disease in a significant number of cases (Rodgers *et al.*, 2007).

The environmental conditions of the affected animal may contribute in having the clinical signs to be more pronounced. Stressful conditions, such as in malnutrition and post-calving, make the clinical signs of BTB to be more pronounced.

Clinical signs may vary depending on the site of localization, which maybe the lungs, liver, spleen, bone, joints and mammary glands but BTB has a long subclinical phase, ranging from a few months to several years. Early infections are often asymptomatic.

BTB is a progressive disease. A progressive loss of condition unassociated with other clinical signs may be the only observable sign especially in cattle with extensive miliary tubercular lesions. The condition of the hair-coat is variable but is usually dull in affected livestock but maybe rough or sleek.

Clinical signs exhibited across most species include emaciation, coughing and associated respiratory problems, swollen lymph nodes, draining sinuses and lameness (observed mainly in carnivores) with the severity of the disease in any individual dependent on the infectious dose, consisting of the number of organisms and number of exposures, route of infection as well as the immune robustness of the individual (Renwick *et al.*, 2007).

Irrespective of the route of infection, it may take years for the clinical signs to develop and the spread of *M. bovis* within the animal is considered a slow process, especially in ruminants and carnivores (Renwick *et al.*, 2007). Most infections are asymptomatic until disseminated lesions develop during the advanced stages and BTB becomes visibly during the “active/clinical” stages where characteristic lesions develop and progress, ultimately leading to death (Renwick *et al.*, 2007). The move from asymptomatic stage to a clinical disease maybe accelerated by repeat BTB infection, poor nutrition, advancing age and super infection due to the presence of other disease agents such as the immunodeficiency viruses in primates (Simian immuno-deficiency virus), cats feline immuno-deficiency virus and humans (human deficiency virus, which tends to exacerbate mycobacterial infections (Renwick *et al.*, 2007).

Anorexia, stunted growth, enlarged lymph nodes and lethargy is observed but the temperature maybe non-specific, fluctuating in most cases. Superficial lymph nodes are enlarged and palpable.

The animal tends to be more docile and sluggish but the eyes remain bright and alert. Usually there is a chronic cough due to bronchopneumonia. This indicates pulmonary involvement. The cough may be suppressed in acute cases, is never loud occurring, and is moist and worst in the morning (Wadhwa *et al.*, 2006). Dyspnoea is more common in advanced or terminal stages. A low grade fever is usually observed, as well as weakness and in appetite.

More specific clinical signs depend on the tissue/organ of localization of the infection post primary dissemination. Tissues and organs commonly affected are those of the respiratory tract, the digestive system, the udder and others.

### **Respiratory Tract**

When tissues of the respiratory tract are affected, as is usually the case when the route of transmission is inhalation, and when the lung is specifically affected, this is referred to as

pulmonary tuberculosis. The granulomatous lesions, also called tubercles, are found in the retropharyngeal, mediastinal and bronchial lymph nodes. Lesions are also found in the lungs to complete the primary complex. Lung lesions may be microscopically or macroscopically visible as encapsulated foci of caseous necrosis. The lung lesions may spread to the visceral and parietal pleura, where they occur in masses and sometimes fuse together. Pulmonary tuberculosis is the most common form of bovine tuberculosis and occurs in 90 % - 95 % of all cases of the disease in cattle (Wadhwa *et al.*, 2006).

### **Gastro-Intestinal-Tract**

Gastro-intestinal tract (GIT) lesions are the second most common form of bovine tuberculosis. The lesions appear as nodules and/or ulcers in the mucosa of the upper alimentary tract, abomasum, small intestine and large intestine. Ulcers develop first in Peyer's Patches in the small intestines. When the GIT is involved, intermittent diarrhea and constipation may be observed but alimentary infection may be primary, as in the case of a calf infected from drinking milk, or secondary, as in swallowing Mycobacteria-laden exudates from the lungs (Cousins, 2001).

### **Reproductive Tract**

Reproductive disorders include uterine tuberculosis in advanced cases and may eventually cause sterility.

Infection of the genitalia may enter either by spread from peritoneum via the oviduct or by the penetration of serosa or by blood stream invasion, in which case the endometrium may be involved in the absence of serous or tubal lesions (Wadhwa *et al.*, 2006).

The tuberculous metritis may result in infertility or recurrent abortion following conception (Wadhwa *et al.*, 2006). Uterine tuberculosis may be peritoneal, with extensive adhesions of the cornua, peritoneum and adjacent organs with multiple abscesses that are glandular with marked hypertrophy of a diffuse or nodular nature, with muco-purulent vulvar discharges and a placentitis similar to *Brucella abortus*. Irregular estrous, and abscesses in testes may be observed (Izatnagar, 2006).

Various forms of tuberculosis are manifested in the udder of bovines. This ranges from a scenario where most of the glandular lobules are replaced by granulomatous tissue to a case where they are clusters of small lobules that have calcified. The resultant mastitis is therefore variable. Some forms of the mastitis are called caseous tuberculous mastitis and tuberculous galactophoritis (Izatnagar, 2006).

In some cases, iatrogenic transmission to the mammary gland has resulted from contaminated intramammary infusions (Cousins, 2011).

### **Other lesions**

Cutaneous, congenital and genital infections have been recorded but are considered to be rare (Cousins, 2001). Sometimes bovine tuberculosis may manifest as small but numerous lesions in a number of organs of the body. This is referred to as generalized or miliary tuberculosis. This follows dissemination by haematogenous spread of the pathogens and subsequent localisation in various organs of the body, followed by development of tubercles at the affected organs (Izatnagar, 2006).

Lesions of bovine tuberculosis may also be found in the vertebrae, ribs and flat bones of the pelvis, especially in young animals. In advanced cases, lesions on central nervous system shows loss of vision, incoordination in gait, partial paralysis, and hyper-aesthesia and circling (Izatnagar, 2006).

In African buffaloes, the most common clinical signs of BTB are debilitation, poor body condition and emaciation. Lagging behind the herd is a crucial phenomenon for African buffaloes affected by BTB (De Vos *et al.*, 2001). The researchers further noted that when an African buffalo herd was being chased by a helicopter, most of the African buffaloes that lagged behind had advanced cases of BTB with disseminated lung lesions or miliary disease. Coughing is a distinct and regular feature in African buffalo herds with a high BTB prevalence (De Vos *et al.*, 2001). The chronic and often subclinical manifestation of BTB in African buffalo makes it virtually impossible to recognize the disease during the sub-terminal phases of the disease on clinical grounds only. Reproductive performance does not seem to be impaired by BTB infections in the African buffalo (De Vos *et al.*, 2001).

### 1.1.5 Diagnosis

#### General

The basis of BTB diagnostic testing is the reactivity to tuberculin, but this reactivity is dependent on the time from infection (Conlan *et al.*, 2012). For the diagnosis of BTB in cattle, both *ante-mortem* and *post mortem* tests are used (Conlan *et al.*, 2012). *Ante-mortem* tests include tests of cellular immunity, such as the tuberculin skin tests, the IFN- $\gamma$  test and lymphocyte transformation test, which are used for BTB diagnosis especially in the early and pre-clinical stages of the disease (Conlan *et al.*, 2012). Other *ante-mortem* diagnostic tests for the diagnosis of BTB are based on humoral immunity, such as ELISA and the lateral flow assay. *M. bovis* PCR probes can be used on tissue cultures and/or live cattle specimens (Conlan *et al.*, 2012). In addition, some *ante-mortem* tests for the diagnosis of BTB that are based on electron nose technology have been developed (Conlan *et al.*, 2012).

#### Historical Background

An analysis of the herd history is very important in the diagnosis of BTB. The herd history should be considerable, comprehensive and cover a relatively long period of time. It should primary focus on the introduction of new animals into the herd and the management system.

#### Clinical signs

As clinical signs are non-specific and depend on the localization site, they need to be carefully analyzed and may at best be used for differential diagnosis.

However, progressive emaciation, prolonged coughing and enlarged superficial lymph nodes may lead one to include bovine tuberculosis in the differential diagnosis. BTB cannot be easily diagnosed using clinical signs because of its long subclinical course and largely non-specific signs (Gumussoy *et al.*, 2007).

#### Pathology

Gross *post mortem* examination at slaughter is also used for diagnosis. Further histopathological examination of the lesion may increase the confidence of the diagnosis. The sensitivity of gross *post mortem* examination is affected by the method employed and the anatomical sites examined (Ameni *et al.*, 2008). Careful examination of at least six pairs of lymph nodes, the lungs and the mesenteric lymph nodes can result in 95 % of cattle with

macroscopic lesions being identified (Ameni *et al.*, 2008). However, gross *post mortem* examination at slaughter maybe insensitive to detecting lesions even though all the principal sites where lesions are to be found are examined (Corner, 1994). This is especially true if there are no visible lesions (NVL). Detailed *post mortem* examination can be used as a gold standard for the determination of the optimal cut-off point for the comparative intra-dermal skin test (Ameni *et al.*, 2008). Furthermore a detailed *post mortem* examination can be used to define BTB disease status in a population (Ameni *et al.*, 2008). For the purposes of BTB detection, a detailed *post mortem* examination, over and above routine meat inspection, includes external inspection and palpation of the seven lobes of the lungs, being the right apical, the right cardiac, the right diaphragmatic, the left apical, the right cardiac, the right diaphragmatic and the accessory lobe (Ameni *et al.*, 2008). This is followed by sectioning each lobe into thin slices, about 2 cm thick each, for the detection of lesions (Ameni *et al.*, 2008). Each of the six lymph nodes, being the mandibular, medial retropharyngeal, cranial and caudal mediastinal, left and right bronchial, hepatic and mesenteric lymph nodes are sectioned to slices, up to 2 mm thick and inspected for the presence of lesions (Ameni *et al.*, 2008).

While most animals infected with *M. bovis* may show lesions typical of BTB at *post mortem* examination, test positive results based on comparison to lesions range 58 % - 75 % for the IFN- $\gamma$  assay, 72 % for culture and 50 % - 90 % for PCR (Meikle *et al.*, 2007).

### **Microscopic examination**

In live animals, lymph nodes aspirates, trans-tracheal aspirates, exudates and milk may be stained with the Ziehl-Neelsen staining technique. Pleomorphic and intracellular acid fast bacilli are observed. The Ziehl-Neelsen stain is subjective to a certain extent and requires skilled and experienced personnel. The sensitivity of this test for the detection of acid fast organism and in particular *M. bovis* from lesions is generally low. The Ziehl-Neelsen staining technique has a low sensitivity for detecting *M. bovis* in bovine and caprine tuberculous lesions and, consequently a low number of positive lesions are identified when using this technique even though a higher magnification is required (Gutierrez *et al.*, 1993). At necropsy, a tentative diagnosis of BTB can be made by the detection of macroscopic lesions typical of BTB (Ameni *et al.*, 2008). The most commonly observed microscopic lesions are characterized by a central necrosis with mineralization surrounded by granulomatous inflammatory response (Ameni *et al.*, 2007).

## Culture

For confirmation of BTB, culture and identification of Mycobacteria from affected tissues or exudates is done. Culture is the gold standard for determining BTB disease status (Ameni *et al.*, 2008). Up to 56 % of animals with gross lesions for BTB are culture positive, while at least 27.3 % and 31.5 % of animals with skin and mesenteric lymph node lesions are culture positive (Ameni *et al.*, 2007). The material which can be used to demonstrate acid fast organism are sputum, faeces, urine, nasal discharges, uterine discharges, milk and other discharges from the lymph. However, Mycobacteria are slow growers and these may take four to six weeks to grow. Bacteriological culture of *M. bovis* in primary isolation media is considered the gold standard for confirmation of the infection in animals (Ameni *et al.*, 2008). Following slaughter, BTB is confirmed by culture of *M. bovis* from the presumptive lesions. Laboratory confirmation of BTB is based on the observation of typical *M. bovis* colony growth and morphology on primary isolation media after incubating at 37 °C for six weeks followed by species identification by PCR. Histopathology and Ziehl-Neelsen staining of tissue specimens containing visible lesions are used as ancillary methods to support bacteriological techniques (Ameni *et al.*, 2008).

After culturing, biochemical tests may be used to detect Mycobacterium species, such as catalase test, Iron uptake test, niacine test, Nitrate reduction test, NaCl tolerance test, Pyrazinamide test, Urease Test and Tween 80 hydrolase test (Izatnagar, 2006).

## Polymerase Chain Reaction (PCR)

The Polymerase Chain Reaction (PCR) is a diagnostic method that can be used for the diagnosis of *M. bovis*. PCR is used to differentiate Mycobacteria of the *M. tuberculosis* complex from non-tuberculous Mycobacteria (Maas *et al.*, 2012). PCR is further used for more specific differentiation of *M. bovis* from other members of the *M. tuberculosis* complex (Maas *et al.*, 2012).

The polymerase chain reaction is a diagnostic method that is particularly useful in the laboratory diagnosis of *M. bovis* in slaughterhouse cases or in species other than cattle such as wildlife. The real-time PCR (Q-PCR) is faster and show more automation possibilities (Parra *et al.*, 2007). Multiplex real-time PCR approach can be used as a high quality *post mortem* diagnostic system applicable directly to bovine clinical specimen and can allow a rapid detection of Mycobacteria's presence (Parra *et al.*, 2007). This makes PCR to be a useful tool in the context of abattoir surveillance programs and granuloma submission programs for bovine tuberculosis

(Parra *et al.*, 2007). Even though PCR sensitivity and specificity are in principle very high, many constraints still preclude its incorporation to the diagnostic routine for the detection of *M. bovis* in milk and other animal specimens.

PCR can be used in the identification of *M. bovis* in fixed formalin tissues with acid fast organisms as long as they have sufficient bacterial DNA (Janice *et al.*, 2002). In most cases, culture results for formalin fixed tissues are negative, even though tests for acid fast organisms maybe positive (Miller *et al.*, 2002). PCR allows the identification of *M. bovis* in such cases, highlighting the PCR's higher sensitivity over the culture method. Furthermore, PCR does not require the presence of viable organism while this is necessary for successful bacterial culture and isolation (Miller *et al.*, 2002).

The major challenge with PCR in the diagnosis of *M. bovis* is the difficulty in extracting Mycobacterial DNA from bovine samples (Parra *et al.*, 2007). This is due to the fact that bovine samples usually have few Mycobacteria in them and the structure of the biological sample itself, which shows strong fibrosis and calcification that hamper access to the DNA to be, detected (Parra *et al.*, 2007).

Spoligotyping is the principal molecular typing technique applied to *M. bovis* isolates recovered from cattle and other animals (Rua-Domenecha *et al.*, 2005). This technique differentiates *M. bovis* strains from other members of the MTBC that infects animals.

Species identification is indispensable for the study of the transmission of Mycobacteria between humans and animals and, together with diagnostics, plays a major role in tuberculosis surveillance and control at the animal-human interface (Michel *et al.*, 2010). PCR-based techniques play a crucial role in species identification for Mycobacteria and are indispensable for their accurate differentiation and molecular epidemiological investigation of tuberculosis transmission (Michel *et al.*, 2010). While biochemical techniques maybe used for differentiation between distinct Mycobacteria species, PCR based techniques have an advantage over them as the former are laborious, time consuming and appear to be erroneous.

There are some challenges to the use of PCR in diagnosis, including the diagnosis of *M. bovis* these include the fact that certain samples may contain PCR inhibitors which could lead to false negative results and the fact that the generation of a vast number of DNA amplicons could

quickly give rise to false positive results (Michel *et al.*, 2010). In addition, PCR requires a sophisticated laboratory and well-trained technicians.

### **Intra-dermal Tuberculin Test**

Internationally, the intra-dermal tuberculin test is commonly used for diagnosis of BTB in cattle (Ameni *et al.*, 2008). This test is the primary diagnostic test for TB and BTB in humans and cattle respectively (Ameni *et al.*, 2008). Various modifications of this test are available but the most common are the single intra-dermal test and the comparative intra-dermal test. This is based on the observed differences of cell mediated immune reaction that occurs when bovine purified protein derivative (PPD) tuberculin is administered intra-dermal in infected and non-infected cattle (Ameni *et al.*, 2008). In infected cattle, the body quickly mounts a CMI reaction which is visualized as a skin reaction, characterized by an increase in skin thickness caused by swelling characterized by edema, heat and sometimes necrosis, at the site of injection (Ameni *et al.*, 2008). In non-infected cattle, there is no such CMI reaction and hence no skin reaction is observed (Ameni *et al.*, 2008). When the bovine PPD is used alone, the test is referred to as a single intra-dermal test but when it is used alongside the avian PPD, the test is referred to as a comparative intra-dermal skin test (CIST) (Ameni *et al.*, 2008). A positive reactor is observed when the bovine PPD shows a stronger reaction, based on the measurable skin thickness, than the avian PPD. According to the OIE recommendation, the difference between the increase in skin thickness following the intra-dermal administration of bovine PPD (B) and the increase in skin thickness following the intra-dermal administration of avian PPD (A), B-A, should be  $> 4$  mm for the animal to be considered to be test positive for BTB (Ameni *et al.*, 2008).

Different cut-off points, other than  $(B-A) > 4$  mm, maybe applied according to a particular country's BTB status and the objective of its BTB control; program (Awah-Ndukum *et al.*, 2012). The OIE cut-off point was established mainly in developed countries for *Bos taurus* cattle, and there is a need for it to be re-evaluated in *Bos indicus* and *Bos taurus* cattle under different environmental conditions, such as tropical, sub-tropical and/or African and Sub-Saharan African conditions (Awah-Ndukum *et al.*, 2012). The performance of the tuberculin skin test can be affected by environmental factors, the prevalence of BTB, hosts factors such as it's immune status, genetics, etc., and the nature of the tuberculin used (Awah-Ndukum *et al.*, 2012). Hence an ideal cut-off point in a specific geographic region with specific environmental conditions may not be as ideal in another geographic region with different environmental conditions (Awah-Ndukum *et al.*, 2012).

The PPD skin test cannot reliably differentiate *Mycobacterium bovis* from non-tuberculous *Mycobacterium* species and therefore false positive results may occur (Liu *et al.*, 2006). The *in vivo* skin test makes it extremely difficult for large-scale epidemiological surveys and retrospective epidemiological analyses, lacks specificity and sensitivity and is associated with errors associated with the mode of administration and the reading of results (Liu *et al.*, 2006; Krishnappa *et al.*, 2006). Other factors that affect the test include the physical condition of the animal like pregnancy, estrus and parasites like liver flukes which must be taken care of for interpretation of the results (Krishnappa *et al.*, 2006).

In bull fighting breeds, the skin test becomes difficult to perform due to the special herd management that substantially differs from other meek breeds (Arenas *et al.*, 2009). Manipulation around the neck are not practical due to the physical dangers that the technician maybe exposed to and the animals will be most reluctant to be handled three days later (Arenas *et al.*, 2009). In such cases, the IFN- $\gamma$  test is recommended.

The BTB status of the herd is important when deciding on whether using the single or comparative intra-dermal test. For herds whose status is known and is negative for BTB, the single intra-dermal test is used.

The test results of the tuberculin skin test maybe influenced by the stage and severity of disease (Ameni *et al.*, 2008). In addition, false negative test results may be obtained, meaning the animal is infected by *M. bovis* but the tuberculin skin test is unable to identify that animal as infected. False negative results may be due to anergy in animals with advanced and/or generalized disease (Ameni *et al.*, 2008). They may also be due to the fact that a reaction to the tuberculin skin test only occurs three to six weeks post infection and hence newly infected animals may not react to the tuberculin skin test (Ameni *et al.*, 2008). Animals that have been subjected to stressful conditions, such as calving within the preceding 4-6 months may also give false negative results (Ameni *et al.*, 2008). The administration of glucocorticosteroids lowers indurations of tuberculin reactions in infected animals and co-infections with viruses such as bovine viral diarrhoea transiently compromises the reaction of the tuberculin test (Ameni *et al.*, 2008). These two phenomenons may cause infected animals to be test negative. Furthermore, a reduced tuberculin reaction may occur if the animal is malnourished, leading to a false negative result.

De-sensitization is a phenomenon whereby there is a depressed skin activity to the second tuberculin injection in natural and experimental infected cattle for some time after the first tuberculin injection reduces the sensitivity of the tuberculin skin test, resulting in false negative results (Ameni *et al.*, 2008). Prior tuberculin test exposure to the Mycobacteria of the *M. avium* complex may lower sensitivity to the tuberculin skin test, as the reaction to avian tuberculin could be high and thus interfere with the interpretation of the results, leading to false negative results (Ameni *et al.*, 2008).

The comparative intra-dermal skin test maybe used repeatedly in non-infected cattle in herds and farms where BTB has occurred (Thom *et al.*, 2004). However, the test is affected by subsequent testing on infected animals, as the procedure for the comparative intra-dermal skin test has consequences for the immune response following infection (Thom *et al.*, 2004). In infected animals, there is a marked reduction in the intensity of the reaction of the skin test, coupled with a marked reduction in the number of animals that would be recognized as reactors (Thom *et al.*, 2004).

### **IFN- $\gamma$ Test**

Another test that can be used for the diagnosis of BTB is the interferon gamma assay. Strategically, the IFN- $\gamma$  assay can be used as means for the early detection and identification of *M. bovis* infected cattle in a herd, ensuring their early removal, which will decrease their infectivity (Gormley *et al.*, 2004). While the tuberculin skin test has become the primary screening test for *M. bovis* infection in cattle, the IFN- $\gamma$  test has become an important ancillary test, especially in developed countries such as New Zealand (Buddle *et al.*, 2006). It can be used for re-testing tuberculin skin test positive animals, to improve specificity and to minimize wastage from slaughtering animals with false positive test results (Buddle *et al.*, 2006).

The IFN- $\gamma$  test can be used in locations of increased risk of infection in parallel with the skin test and for the re-testing of tuberculin skin test positive animals for pre-movement testing (Buddle *et al.*, 2006). The test can further be used in problem herd to identify *M. bovis* infected animals that do not respond to the skin test (Buddle *et al.*, 2006).

The Bovigam test is a trademark variation of the IFN- $\gamma$  test developed by Prionics ([www.prionics.com](http://www.prionics.com)). It is a rapid in vitro blood based assay of cell mediated responses to *M. bovis* PPD tuberculin for the diagnosis of bovine tuberculosis infection in cattle, sheep, goats, buffalo, bison and other bovidae ([www.prionics.com](http://www.prionics.com)) it is widely used as an ancillary test

to the tuberculin skin-test. Tuberculin PPD antigens are presented to lymphocytes in whole blood culture.

The Bovigam test is performed in two stages. In the first stage, the blood samples are incubated overnight with antigen (e.g. tuberculin PPD) to stimulate the lymphocytes to produce IFN- $\gamma$ . The production of IFN- $\gamma$  from the cells is then detected using monoclonal antibody-based sandwich enzyme immune assay (EIA). Lymphocytes from cattle not infected with *M. bovis* do not produce IFN- $\gamma$  therefore, detection of IFN- $\gamma$  correlates to *M. bovis* infection ([www.prionics.com](http://www.prionics.com)). Animals infected with *M. bovis* can be identified by measuring the cytokine interferon gamma against bovine and avian tuberculin.

Using the gamma interferon assay as an ancillary test for BTB diagnosis has improved the sensitivity of BTB testing (Wood *et al.*, 1991). The test is also credited with better detection of early *M. bovis* infections relative to other tests, such as the intra-dermal skin test (Neill *et al.*, 1994). In this test, heparinised blood samples are used to detect the presence of the interferon- $\gamma$  in the blood after in vitro stimulation. However, it has a problem of false positive reactions due to the cross-reactive nature of the antigen preparation used (Wood *et al.*, 2002). False positive results were obtained when testing for BTB in free-ranging African buffaloes using the gamma interferon assay and this was attributed to the sensitization of the animals with environmental Mycobacteria (Michel *et al.*, 2009). This can be overcome by using a comparative assay in which an animal's IFN- $\gamma$  response to bovine PPD and avian PPD are compared (Wood *et al.*, 2002). Furthermore, the use of fortuitum PPD in detecting non-specific sensitization in cattle and African buffaloes has allowed improved test specificity in uninfected herds and populations. The specificity can be increased by differentiating the immune response caused by environmental Mycobacteria and true bovine reactions through the addition of fortuitum protein as a stimulating agent in the IFN- $\gamma$  assay (Grobler *et al.*, 2002; Michel, 2002; Michel *et al.*, 2011). The IFN- $\gamma$  test is also recommended in animals that are difficult to handle or capture or both, such as excitable cattle, bull fighting breeds and wildlife, although the frequency of the testing should be increased (Arenas *et al.*, 2009). The IFN- $\gamma$  has been proven to be a very specific test, discriminating easily the sensitization of the lymphocyte with bovine and avian PPD in bovine blood (Arenas *et al.*, 2009).

It has been observed that parallel interpretations of the gamma interferon assay and the intra-dermal skin test exceeds their individual diagnostic sensitivities (Whipple *et al.*, 1995).

However, animals can circulate IFN- $\gamma$  in response to non-specific immune response to infections other than *M. bovis*. Hence an animal could be circulating IFN- $\gamma$  due to other infections, abscesses or parasites even before being stimulated by *M. bovis* PPD, hence a mere presence of circulating IFN- $\gamma$  may not be significantly linked to *M. bovis* infection. Hence for circulating IFN- $\gamma$  to be linked to *M. bovis*, the OD-reading should be above 0.38 (Michel *et al.*, 2011b).

Furthermore, the performance of the IFN- $\gamma$  can be influenced by other factors such as a recent tuberculin skin test and the length of time delay between collection and processing of blood samples (Gormely *et al.*, 2004). A delay in processing of the blood samples from cattle could significantly impact on the outcome of the IFN- $\gamma$  gamma assay, resulting in a change of the IFN- $\gamma$  status of the animal (Gormely *et al.*, 2004).

Several modifications of the IFN- $\gamma$  assays are being developed to improve specificity, such as by altering the cut-off point or using specific antigens present in virulent Mycobacteria such as the 6 kDa early secreted antigenic target (ESAT-6) and 10 kDa culture filtrate protein (CFP-10) (Buddle *et al.*, 2006).

Lauzi *et al.* (2000) investigated the specificity of the IFN- $\gamma$  test in cattle herds in Italy that had been certified free of BTB, finding it to be 88.8 % after a single test and 95.4 % after a double sampling scheme. However, Ryan *et al.* (2000), found a slightly lower specificity of the test at 85 %, with a sensitivity of 93 %. The accuracy of a diagnostic test is measured by the relationship between sensitivity and specificity, which determines the false-positive and the false-negative proportions (Rua-Domenech *et al.*, 2005). None of the current tests being used to diagnose BTB, including the tuberculin skin test and the IFN- $\gamma$  assay allow for a perfectly accurate determination of the *M. bovis* infection status of cattle (Rua-Domenech *et al.*, 2005). In his investigations, Lauzi *et al.* (2000) found compelling evidence that the specificity of the IFN- $\gamma$  assay could be related to the animal's interaction with environmental Mycobacteria and/or ageing. The researchers identified the need for test procedures to utilize more specific antigens and different reaction thresholds in order to reduce the percentage of non-specific bovine reactors, especially in herds considered free of BTB (Lauzi *et al.*, 2000).

### **The Enzyme-Linked Immunosorbent Assay (ELISA)**

The Enzyme-Linked Immunosorbent Assay (ELISA) can also be used in diagnosis but only in advanced cases as the production of anti-bodies commences at the advanced stages of the

disease. ELISA is a rapid, simple and low cost test that measures antibody titres to *M. bovis* and maybe useful in the diagnosis of TB (Liu *et al.*, 2006). ELISA may complement tests of cellular immunity in anergic cattle but, as a humoral test, it has limited use in the diagnosis of BTB in cattle as titres are inconsistent and rise only in the later stages of the infection, although in the deer, titres may rise earlier in the course of the disease and are much more predictable (Liu *et al.*, 2006). Its sensitivity and specificity is relatively low (Liu *et al.*, 2006). However, Veeregowda *et al.* (2005) claims that the ELISA has the ability to detect antibody levels several hundredfold lower than most serological test but needs to be improved by using anti-IgM and anti-IgG conjugates, ignoring the cross reactivity between the light chains of IgG and other immunoglobulin types.

Besides, *Mycobacterium bovis* is an intracellular parasitic bacterium and hence it exhibits large individual differences in antibody reactivity and has a wide antibody reactivity spectrum, creating a problem in coming up with a single suitable antigen or combination of antigens for the use in the ELISA test (Liu *et al.*, 2006).

Liu *et al.* (2006) compared the ELISA test and the PPD skin test and found that the ELISA gives a 68.7 % positive ratio for the PPD skin test positive sera and 100 % negative ratio for the PPD skin test negative sera. They recommend that the ELISA be used either as a supplementary method for the PPD skin test or as an efficient tool for epidemiological surveys of the cattle and wild animals (Liu *et al.*, 2006). De Kantor *et al.* (2005) believes that at best, ELISA may play a modest role in the screening at the herd level or in the detection of advanced disease.

The weak humoral immune response inherent to natural infection, the wide cross reactivity with immunodominant mycobacterial antigens and the genetically controlled diversity of individual responses to mycobacterial epitopes are largely responsible for the poor correlation observed between the results of serology and those observed by bacteriology (Veeregowda *et al.*, 2005). Furthermore, in field trials, the ELISA lacks specificity, due to the fact that cattle are exposed to many other *Mycobacteria* in the environment leading to antibody responses to shared antigens of *Mycobacteria* species, resulting in a high number of false positives (Veeregowda *et al.*, 2005).

## **Other Methods**

Other tests that maybe be used for diagnosis of BTB include the half automated radiometric BACTEC 460 TB system, which is one of the widely used culture techniques among commercial

systems (Gumussoy *et al.*, 2007). Chest radiography may be very helpful in TB diagnosis of small domesticated animals (Izatnagar, 2006). The short thermal test is performed on animals with a rectal temperature of less than 39 °C (Izatnagar, 2006). Intradermal tuberculin is injected subcutaneous and the temperature is monitored. If the temperature at 4, 6 and 8 hours after injection rises above 40 °C, the animal is classified as a positive reactor (Izatnagar, 2006).

### **Differential diagnosis**

During *post mortem* or at necropsy, the granulomatous lesions can be confused with others caused by infectious agents such as fungi, staphylococci, and *Arcanobacterium* and *Actinobacillus* species. Hence there is a need for further testing, with the Ziehl-Neelsen staining and a confirmatory test such as culture (Corner, 1994).

The clinical signs may be confused with those caused by traumatic reticulitis (especially lesions and ulcers in the gastro intestinal tracts as well as in the lung parenchyma), chronic pasteurellosis, aspiration or foreign body pneumonia, *Actinobacillosis*, *Arcanobacterium pyogenes* infection and *Corynebacterium pseudotuberculosis* infection (Blood *et al.*, 1992). The mastitis can be confused by other clinical mastitis conditions caused by other causes of mastitis. Hence for a definitive diagnosis of bovine tuberculosis, the clinical signs cannot be used in isolation as there is no pathognomonic clinical sign (Blood *et al.*, 1992).

#### **1.1.6 Control of Bovine Tuberculosis**

The aim of controlling bovine tuberculosis is to eventually eradicate the disease from a country. However, eradication on a national level is not an easily achievable target and requires a combination of policies and strategies, such as the test and slaughter policy described below. Accurate detection and removal of infected cattle, using immunodiagnostic tests such as the comparative intra-dermal skin tests, are the basis of a control strategy for BTB (Cousins, 2001). BTB infection in a cattle population is usually chronic and can remain subclinical for a long period, with infected cattle becoming infectious long before they exhibit any clinical signs hence effective *ante-mortem* surveillance must primarily rely on the detection of infected cattle at an early stage by the use of sensitive immunodiagnostic tests (Ameni *et al.*, 2008). Controlling or eradicating BTB is a strategy for eradicating human tuberculosis caused by *M. bovis* (Liu *et al.*, 2006).

BTB control strategies that are based on systematic and regular tuberculin skin testing of cattle herds, followed by the compulsory removal of all test positive animals, movement restrictions of infected herds and an effective surveillance system at abattoirs has proven to be successful in eradicating BTB from other regions and/or countries (Ameni *et al.*, 2008).

In the test and slaughter policy, animals in a particular area or region or even country are tested for bovine tuberculosis and all test positive animals are slaughtered (Conlan *et al.*, 2012). The testing of the animals should be compulsory and at designated regular intervals, e.g. annually. Sometimes, and in addition, only animals which show suspicious clinical signs are tested. Animals are then kept in quarantine, depending on the outcome of the results. Use of quarantine is also extended when new animals are to be introduced into the population. Attached to this method is the control of animal movement as well as a viable system of animal identification. The co-operation of farmers is essential as they have to understand that the destruction of their cattle is for the benefit for all. A compensation scheme for destroyed animal is helpful to get farmers on board. There are several challenges associated with this method, paramount amongst them being the huge amount of resources, financial and human, required (Conlan *et al.*, 2012).

For pedigree and other valuable livestock, such as those which may have sentimental value, they may not be necessarily destroyed but they are isolated from the rest of the herd. If these animals are exotic species in zoological collections, they may be treated with isonicotinic-hydrazine, after permission has been granted by the relevant veterinary authorities. However, the treatment is just a temporary measure and the animal is eventually slaughtered. This occurs in very rare circumstances (Conlan *et al.*, 2012).

It should be noted that the test and slaughter method, and the accessory activities discussed above, are less effective where wildlife reservoirs of bovine tuberculosis exists (Buddle, 2004). Wildlife reservoirs of *M. bovis* have caused major challenges in the eradication of the disease from domestic animals.

In preventing human infection from meat, specifically the viscera or internal organs, the head and meat cuts containing lymph material, it is recommended that cattle should undergo both *ante-* and *post mortem* inspection. All suspicious cases should be confirmed in the laboratory and all positive carcasses should be completely destroyed. In a carcass, the internal organs and meat cuts containing lymph nodes pose a high risk compared to ordinary meat cuts (Conlan *et al.*, 2012).

Thorough cooking of meat is also recommended as the bacteria are heat labile (Van der Merwe *et al.*, 2009).

Milk must be pasteurized before being consumed unless it comes from animals that are certified to be negative for bovine tuberculosis. Care must be taken during the pasteurization process to ensure that it is effective. Milk with high somatic cell content or one that contains pus or is sour or is incompletely clarified, the effectiveness of the pasteurization is not comprehensive.

Infected premises need to be disinfected. Good hygiene practices, such as thorough cleaning, are indicated. Sterilization should be attempted by using heat, UV light and other disinfectants.

Movement control of livestock and wildlife, especially from herds or populations that are known to be infected with *M. bovis* is a crucial factor in the control of BTB (Ameni *et al.*, 2008). In a cattle herd where an eradication program is in place, BTB is unlikely to persist in the absence of an anergic animal and/or an external source of infection (Barlow *et al.*, 1997). However, in the United Kingdom, BTB persisted in about 38 % of the herds despite a BTB control program and movement control in place, indicating that such programs cannot always be 100 % effective (Conlan *et al.*, 2012). Conlan *et al.* (2012) attributes this persistence in BTB in the presence of eradication and movement control programs to infected cattle being missed by tuberculin testing.

In a herd infected with the same strain of *M. bovis*, *ante-mortem* and immunodiagnostic parameters change dramatically, suggesting that several tests, such as the tuberculin skin test, culture and the IFN- $\gamma$  assay, are needed simultaneously for a faster control of infection at herd level (Meikle *et al.*, 2007).

### **Other Control Strategies**

Instead of the test and slaughter methods, a test and segregate method maybe used, where domestic animals are tested and all reactors removed and physically separated from non-reactors (Barlow *et al.*, 1997). The infected animals are quarantined while the non-reactors are repeatedly tested and subsequent reactors are removed (Barlow *et al.*, 1997). This method maybe used in the early stages of an eradication program before switching to the test and slaughter method (Barlow *et al.*, 1997).

Sanitation and disinfection may reduce the spread of *M. bovis* within a herd, with 5 % phenol, highly concentrated iodine solutions, glutaraldehyde and formaldehyde being effective disinfectants.

Rodent control is advisable in infected farms as mice and voles have been experimentally infected with *M. bovis* and may shed the bacteria in faeces which may come to contact with domestic animals (Conlan *et al.*, 2012).

Culling in infected wild animals reduces the animal density which can decrease transmission but the culling may increase the dispersal of the remaining members of the species (Barlow *et al.*, 2012). Furthermore, prohibition of supplementary feeding and baiting, a practice where hunters feed wild life, can decrease the transmission of *M. bovis* in feeding areas. In farming enterprises in the vicinity of wildlife, physical barriers maybe used around feed for domestic cattle, such as hay, to prevent wildlife feeding on it (Barlow *et al.*, 2012). Bio-security in such scenario's prevent wildlife interacting with domestic animals and hence reduce the transmission of *M. bovis* between the two (Barlow *et al.*, 2012).

Antimicrobial treatment is prohibited in most countries due to the risk of shedding the pathogenic organism, potential hazards to humans and the potential to drug resistance, but if it has to be done, it must be long term and clinical improvement can occur without bacteriological cure.

### **BTB Control Program in Swaziland**

Several countries have a BTB surveillance program in place. In Swaziland, the BTB surveillance program focuses mainly on dairy animals and an abattoir surveillance program in only one slaughterhouse, the Swaziland Meat Industries (Annual Report, 2011). All dairy animals are tested annually for BTB using the comparative intra-dermal skin test. Positive animals are re-tested 90 days after the positive result has been received, and those that remain positive are destroyed. Cattle meant for export are also tested and positive animals are not only denied export status but are also destroyed. Animals that are reported sick with clinical signs similar to BTB are also tested and if found positive are also destroyed. Routine meat inspection, including the detection of lesions typical of BTB followed by microscopical examination, is carried out the Swaziland Meat Industries for each animal that is slaughtered. Between 2003 and 2011, 189 cases of BTB have been confirmed at SMI (Annual Report, 2011).

## Vaccination for BTB

Vaccinating for BTB could be beneficial, especially when used in conjunction with other methods to control BTB in cattle and in some wild animals such as the badger, as it could reduce the prevalence, incidence and the rate of spread of the disease in the animal population ([www.defra.gov.uk](http://www.defra.gov.uk)).

*M. bovis* bacillus Calmette-Guerin (BCG) has been used worldwide for vaccination against human tuberculosis, even though it has been shown to have variable efficacy (Murphy *et al.*, 1996). Experiments have indicated that in cattle, BCG vaccination reduces the progression, severity and excretion of BTB as well as reducing the transmission amongst animals ([www.defra.gov.uk](http://www.defra.gov.uk)). Other studies in Mexico and Ethiopia indicate that the protective effect of vaccinating cattle with BCG vaccine was between 56 % and 68 % ([www.defra.gov.uk](http://www.defra.gov.uk)).

Vaccination results in the development of mainly cell-mediated immunity in an animal. Due to the fact that alveolar macrophages have no immunological memory, the bacilli must multiply first and produce antigenic products which can be recognized by the lymphocytes, hence vaccination only stops the development of a clinical disease but does not prevent infection (Dannenbergh, 2001).

In cattle, BCG induces a significant level of protection against *M. bovis* infection when cattle are experimentally challenged, (Buddle *et al.*, 2004). In field trials, the results are not as encouraging mainly due to an overwhelming challenge in real life infection which outstrips the immune response, prior exposure of calves to environmental mycobacterium which may prevent or mask the development of protective immunity and very high immunizing doses of BCG (Buddle *et al.*, 2004). Even though BCG vaccination is safe and relatively cheap, it further has a variable efficacy and may induce positive reactions in the tuberculin skin test. The route of administration is also a challenge as *M. bovis* is a pathogen that is generally transmitted via the mucosal surfaces and it is possible that better protection may be induced when BCG is delivered by the mucosal route (Buddle *et al.*, 2004). However, it is believed that BCG vaccine is likely to be used in future as a vaccine in cattle, either on its own or in combination with other antigens (Widdison *et al.*, 2009). Studies to demonstrate the safety and efficacy of the BCG vaccine were successfully conducted by the Animal Health and Veterinary Laboratory Agency of the Department of Agriculture in the United Kingdom and the latter consequently made an application for marketing authorization with that country's Veterinary Medicine Directorate as recent as the beginning of 2012 ([www.defra.gov.uk](http://www.defra.gov.uk)).

## 1.2 Problem statement

The sporadic occurrence of positive BTB carcasses on meat inspection is indicative of an underlying problem of BTB in the respective dip tanks. The passive management of cattle in dip tanks in Swaziland, especially inadequate clinical veterinary services and consultation, promotes the prevalence of chronic zoonotic diseases such as BTB in cattle populations. Insufficient provision of clinical veterinary services, inadequate BTB control measures, a high rate of cattle movement into and out of kraals/dip tanks and a lack of a comprehensive and effective BTB mitigating policy within kraals/dip tanks have contributed to the significant prevalence of BTB. Milk pasteurization is not comprehensively carried out on all milk consumed and inadequate veterinary public health measures lead to at least an estimated 90 % of all consumed meat, including visceral organs not subjected to *ante-mortem* and *post mortem* inspection. The human population of Swaziland is currently enduring the HIV/AIDs epidemic and a significant number of people are already immune-compromised or will soon be immune-compromised. The current program of controlling bovine tuberculosis is no longer effective or being properly implemented.

## 1.3 Hypothesis

Bovine Tuberculosis is expected to be present in cattle herds in Swaziland at a significant rate and high enough to constitute a zoonotic health threat to humans.

## Chapter 2

# Materials and Methods

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### 2.1 Study population

#### 2.1.1 Selection of Dip tanks of Study

##### General

There were an estimated 616 459 heads of cattle in Swaziland distributed amongst 413 dip tanks at the end of 2010 (Annual Report, 2010). The Swaziland Meat Industries, SMI, is the biggest abattoir and the biggest buyer of cattle in the country, and exports meat to both regional and European markets. To cattle farmers in the country, it is also a lucrative market for their cattle, with agents distributed all over the country with the sole purpose of buying cattle for slaughter for the abattoir. Consequently, cattle slaughtered at SMI comes from all over the country, providing a true representative of the cattle population in the country.

In addition, each animal slaughtered at SMI undergoes a full *ante-mortem* and *post mortem* examination, and all animals with BTB-like lesions are further sampled and tested for the presence of acid fast organism. All findings are recorded with the records being kept for a period of two years.

No such other abattoir in the country has an equivalent or better system and/or records, making SMI the most suitable place for studying bovine tuberculosis in Swaziland.

##### Examination of Record

Slaughter records at the Swaziland Meat Industry's (SMI) export abattoir were examined, for the period 1 July 2009 to 30 June 2010. The daily slaughter records were examined, which record all pathological findings on organs and carcasses at the kill floor, the condemnation report, which records carcasses and organs that have been condemned and the reason for such condemnation, the slaughter sheet which has a comprehensive information on all animal slaughtered, the Daily Book at the laboratory and the Meat Hygiene Laboratory Record, which records laboratory findings on samples taken to the laboratory after each slaughter.

## Traceability Exercise

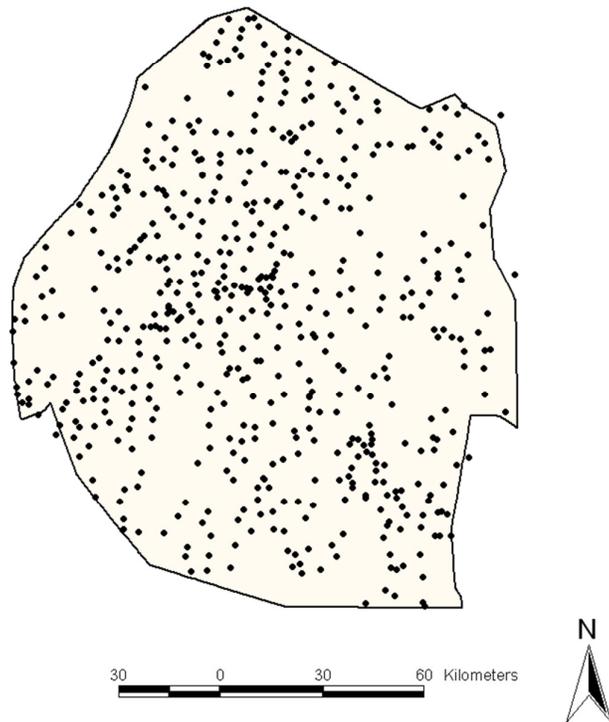
All carcasses that tested positive for BTB, identified by tuberculous lesions at *post mortem* inspection and testing positive for acid fast organism when tested using the Ziehl-Neelsen technique, during the study period were identified. A carcass had to be marked positive for BTB in all the examined records for it to be included in the study.

Using the country's traceability system, each carcass was traced back to its respective dip tank of origin. This was done by noting the carcass's serial number and date of slaughter. This led to identifying the ear tag number of the animal from which the carcass was derived. The ear tag number traces back to the Stock Removal Permit (SRP), which is the document that authorized the movement of the animal from its respective dip tank to the abattoir. The SRP has the name and number of the dip tank from which the animal originated from, allowing for the identification of the dip tank of origin.

## Selecting Dip Tank of Study

The traceability exercise revealed that during the 12 months period from 1 July 2009 to 30 June 2012, carcasses that were BTB positive on *post mortem* examination and subsequent laboratory testing by staining for acid fast organisms, were derived from cattle that came from sixteen dip tanks. These sixteen dip tanks, out of a total of 413 dip tanks in the country shown in **Figure 2**, were selected and designated the dip tanks of study (**Figure 3**). These were Croydon, TM-Corporation, EIEI-Corporation, Langkraal, Matata, Thunzini, Piennarr, Lumphala, Mkhwa, Mtilane, Sigombeni, Elukwatini, Metulo, Lovunga, Mpofu and Mafutseni (**Table 2; Figure 3**).

## DIPTANKS IN SWAZILAND



**Figure 2** A map of Swaziland indicating all dip tanks in the country

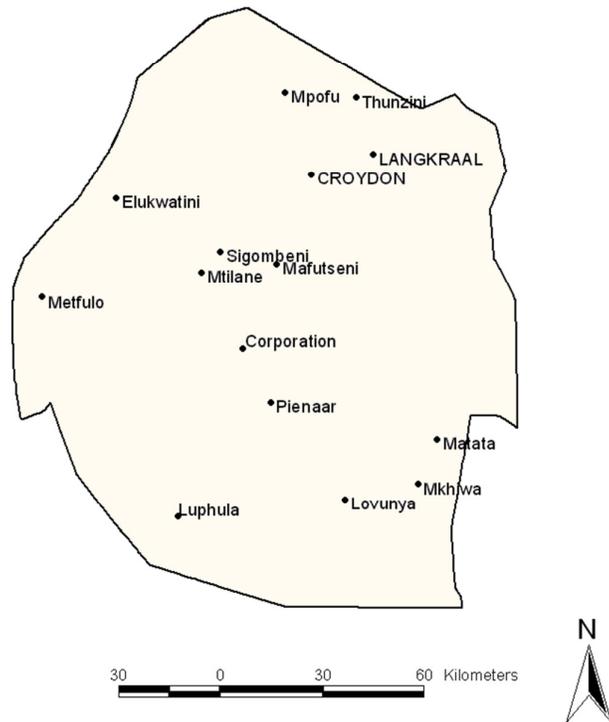
The 16 dip tanks of study were almost evenly distributed in the country and they came from the four regions of the Swaziland, being Hhohho, Manzini, Shiselweni and Lubombo.

**Table 2** List of dip tank that had a BTB positive carcass on meat inspection at SMI between 1 July 2009 and 30 June 2010. Letters in parentheses denotes the district from which dip tank is located.

	<b>Number</b>	<b>Name</b>	<b>Place</b>	<b>No of cattle</b>	<b>Comments</b>
1.	428	Sigombeni	Sigombeni (M)	1258	SNL
2.	602	Pienarr	Kaphunga (S)	1164	SNL
3.	749	Luphala	Nhlangano (S)	874	SNL
4.	368	Mkhiwa	Sithobela (L)	1057	SNL
5.	458	Metulo	Mpuluzi (H)	1017	SNL
6.	867	Elukwatini	Motshane (H)	954	SNL
7.	29	Mpofu	Buhleni (H)	875	SNL
8.	197	Matata	Lubuli (L)	432	Private Farm
9.	209	Langkraal	Ngomane(L)	379	SNL
10.	192	Lovunga	Maloma (L)	781	Private
11.	201	Thunzini	Ntshaneni (L)	883	SNL
12.	432	Mafutheni	Mafutheni (M)	236	Private Farm
13.	509	TM-Corporation	Sidvokodvo (M)	465	Private Farm
14.	402	Croydon	Mliba (M)	1594	SNL
15.	439	Mtilane	Boyane (M)	1024	SNL
16.	509	EIEI-Corporation	Sidvokodvo	465	Private farm

M-Manzini  
 H-Hhohho  
 S-Shiselweni  
 L-Lubombo

## DIPTANKS SAMPLED



**Figure 3** A map of Swaziland indicating the dip tanks of study

### 2.1.2 Description of the study population

The cattle population in the 16 dip tanks of study was 12 993 heads of cattle, which were mainly of the indigenous Nguni breed and/or hybrids of the Nguni breed. The number of cattle per dip tank varied greatly, ranging from 236 heads of cattle in one dip tank to 1596 heads of cattle in another dip tank. In total, there were 504 herds in all the dip tanks, with the number of herds per dip tank ranging from one to about 120. There were more heads per dip tank in the communal dip tanks than in the dip tanks found in title deed farms. The number of cattle in a herd was also variable, with some farmers owning as little as four heads of cattle while others had as much as 256 heads of cattle (**Table 3**).

**Table 3** Description of the study population

	Total	Range amongst dip tanks	Average
Cattle population	12993	-	-
Dip tanks	16	-	-
Herds	504	1-120	69
Herd size	Variable	4-256	30-50

### Calculating Sample Size

The following formula was used to estimate the sample size

$$n = \frac{t^2 \times P (1 - P)}{m^2}$$

Where  $n$  = required sample size

$t$  = confidence level at 95 % (Standard value of 1.96)

$p$  = estimated prevalence of BTB in each dip tank

$m$  = margin of error at 5 % (standard value of 0.05)

$p$  was estimated at 5 % (0.05) in each of the dip tank of study

$$n = \frac{1.96^2 \times 0.05 (1 - 0.05)}{0.05^2}$$

$$n = 73$$

### Contingency

The sample size was increased to 100 animals per dip tank to account for contingencies such as the expected non-return of cattle for the reading of the test.

#### 2.1.3 Identification of animals to be tested

Systematic sampling was used to select cattle to be tested in the study, where  $k$ , the sampling interval was 10 and every tenth animal was selected. In each dip tank of study, the cattle were put in a crush pen, with cattle belonging to the same kraal being put at the same time. Every tenth animal in the crush pen was selected for testing for BTB, on condition that it is an adult animal (above 30 months old). If it was not an adult animal, the next animal was then selected.

Furthermore, every kraal in the dip tank had to be represented by at least one animal. At least 10 % of the animals presented at a dip tank were selected for testing. In dip tanks that had a high number of carcasses testing positive for BTB at SMI during the period of study, such as at Co-corporation, Mafutheni and Mpofu, slightly more than the 10 % of the animals were selected for testing. There is a possibility of a slight sampling bias that might have been introduced when some owners in some dip tanks decided which animals fulfilled the inclusion criterion and had to be tested (Tschopp *et al.*, 2009).

#### **2.1.4 Introduction of study to stakeholders**

The Department of Veterinary and Livestock Services, which is the custodian of all livestock, was informed of the study and a written approval obtained authorizing the study. Secondly, veterinary officials responsible for the dip tanks of study were officially informed and the study carefully explained to them. Thirdly, each of the dip tanks of study was visited by the researcher where he met farmers and animal health technicians and introduced the study prior to the commencement of the study. During these meetings, farmers and technicians were allowed to question and/or seek clarification on any aspect of the study that they did not understand.

## **2.2 Comparative intra-dermal skin test**

### **2.2.1 Restrain of animals and preparation of injection sites**

Cattle were tightly but neatly packed in a crush pen with the animal to be tested further tied on the horns by a rope. The use of electrical or chemical forms of restrains was strictly forbidden, while farmers were also encouraged to limit the use of excessive force during the restraint of cattle to be tested.

### **Shaving and disinfection**

Two areas in the neck region, which were about 12-15 cm apart, were shaved. Shaving was done by one of two research assistants, who are animal health technicians by training. While in most cases, shaving was done on the right side of the neck, a few animals were shaved on the left due to their orientation and ease of restrain as well as the accessibility of the neck in the crush pen. The selected site was manually shaved using *Lion* and *Minora* razor blades. The shaved areas were wiped dry and any debris, such as hairs, removed. The anterior shaved area was designated as “Bovine” while the posterior shaved area was designated “Avian” in all the cattle tested.

## **Measurement of skin thickness**

After shaving, the skin thickness in the injecting sites designated “bovine” and “avian” were measured using a set of calipers and the thickness recorded for each animal tested. One set of calipers was used in all the cattle tested in all the fifteen dip tanks of study. Skin thickness for the “Bovine” and “Avian” injection sites for each animal was recorded accordingly.

### **2.2.2 Injection of Bovine PPD and Avian PPD**

At all material times, the bovine and avian tuberculin was kept either in a refrigerator or on ice packs in a cooler box. In each animal, 0.1 ml volume of 0.1 mg Bovine PPD was inoculated intra-dermal into the skin of the “Bovine” injection site area using 1 ml disposable tuberculin syringes and a 25 gauge needles, with the tuberculin forming a pea shaped swelling in the skin. This was followed by the inoculation of 0.1 ml volume of 0.05 mg Avian tuberculin into the skin of the “avian” injection site.

### **Observation of injection sites**

After 72 hours, the cattle being tested were returned to the crush pen for reading of the tuberculin test. The “bovine” and “avian” injection sites were carefully examined, noting the presence of swellings and any other observation. Each observation, including the characteristics of the swelling, was recorded for each animal.

### **2.2.3 Reading and interpretation of the results obtained in the intra-dermal skin test**

The skin thickness for each “bovine” and “avian” injection sites was measured and recorded again 72 hours after injection of tuberculin. In cases where there was excessive skin swelling such that measuring skin thickness was not possible, skin thickness was denoted as immeasurable.

Calculation of the difference in skin thickness between the bovine and avian injection sites.

The initial skin thickness for the “bovine” and “avian” injection sites before inoculating tuberculin was denoted B1 and A1 respectively. The skin thickness for the respective injection sites after 72 hours from the injection of tuberculin was denoted B2 and A2, respectively. A change in the skin thickness in the “bovine” injection site was designated as  $\Delta B$  and calculated as B2-B1.

$$\Delta B = B2-B1$$

A change in the skin thickness in the “avian” injection site was designated as  $\Delta A$  and calculated as  $A2-A1$ .

$$\Delta A = A2-A1$$

$\Delta B-\Delta A$  is the overall change in the skin thickness at the bovine injection site when compared with that of the avian injection site. This is denoted as the Bovine Bias and was used to determine whether an animal was positive or negative for BTB.

A positive result for BTB was defined as follows:  $\Delta B-\Delta A \geq 4$  mm. A negative result for BTB was defined as follows:  $\Delta B-\Delta A \leq 2$  mm. In cases where  $\Delta B-\Delta A$  was between 2 mm and 4 mm, it was regarded as inconclusive.

If  $\Delta B-\Delta A > 0$ , the overall change in the skin thickness at the bovine injection site was higher than that of the avian injection site.

### **2.3 Slaughter, *Post Mortem* Examination and Culture**

Farmers with test positive cattle for BTB were requested to donate animals to the study for slaughter and *post mortem* examination. Two farmers donated cattle to the study, one donating seven heads of cattle as part of a culling process that removed all the animals that had tested positive for BTB. A second farmer donated another animal that was slaughtered. The seven head of cattle were slaughtered at the Swaziland Meat Industries followed by routine *post mortem* inspection by meat inspectors. The eighth animal was slaughtered at the farm of origin.

#### **Culture of *M. bovis***

##### **A. Sample collection**

Tissue samples were collected as aseptically as possible during *post mortem* of the eight heads of cattle that were sacrificed. The same team collected the tissue samples which consisted of lymph nodes and lung tissues both with and without macroscopic lesions.

##### **B. After collection, tissue samples were sent to the BSL2+ laboratory where they were stored at -20 °C in a designated freezer. Before processing the frozen tissue samples were allowed to defrost in the biohazard cabinet in the BSL2+ laboratory on the day of processing.**

- C. The samples were examined macroscopically and findings regarding the size, abnormal consistency, colour, smell and the presence or absence of visible lesions were recorded.
- D. All fat was removed and  $\pm 5$  gm of tissue was cut into small pieces ( $\pm 0.5$  cm). If visible lesions or abnormalities were observed, they formed part of the sample processed.
- E. An impression smear was made as described under Ziehl-Neelsen staining method.
- F. Specimen was placed into a sterile homogenizing jar, while the rest of the sample was discarded into the waste container.
- G. The specimen was covered in the homogenizing container with distilled, sterile water. The sample/water mixture was homogenized in an automatic homogenizing apparatus for until fully homogenised.
- H. The homogenate was divided into 2 x 15 ml-centrifuge tubes, 7 ml each and the remaining homogenate was poured into a 15 ml centrifuge tube for storage at  $-20$  °C in the BSL2+ lab as reference sample. The rest of the homogenate was discarded in a waste container (with 10 % Jik) inside the biohazard cabinet until autoclaved. Any remaining tissue was placed in a sealed plastic bag and placed in a biohazard bag and autoclaved.
- I. Sample was decontaminated by using 7 ml  $\pm 0.5$  ml 2 % HCl (final concentration 1 % HCl) to one tube and 7 ml  $\pm 0.5$  ml 4 % NaOH (final concentration NaOH 2 %) to the other.
- J. Sample was left for 10 minutes and then centrifuged at 3 500 rpm for 10 minutes.
- K. The supernatant was poured off and then neutralized with 7 ml  $\pm 0.5$  ml distilled sterile water before being centrifuge again for 10 minutes at 3 500 rpm.
- L. Most of the supernatant was poured off keeping approximately 1 ml.
- M. Mix the pellet was mixed with the back of an inoculation loop and the loop was discarded into beaker with 10 % Jik.
- N. One loop full of each of the pellets was spread evenly onto 2 LJ- pyruvate and 1 LJ-glycerol slants, respectively, and incubated at  $37 \pm 1$  °C for 10 weeks (a total of six slants per sample).
- O. A second smear was prepared from the remaining sediment for ZN staining.
- P. All materials used (e.g tweezers, scissors and loops) were placed in a beaker/container with 10 % Jik. The remaining tissues were placed in container and autoclave before discarding.

## **2.4 Interferon Gamma Assay (IFN- $\gamma$ or Bovigam 1G)**

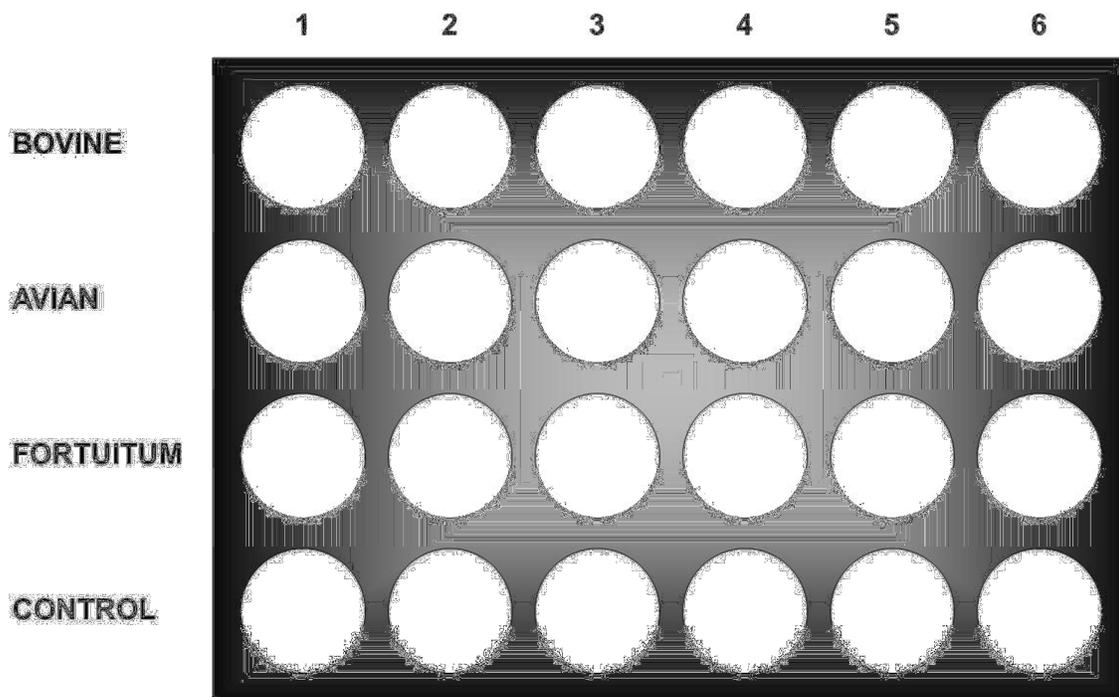
### **2.4.1 Collection and stimulation of whole blood samples**

Cattle were bled shortly before inoculation with tuberculin. Blood collecting tubes containing heparin as an anti-coagulant were clearly marked with the identity of each animal. Each animal

was bled by veno-puncture of the jugular vein using 18G vacutainer needles whereby 7-9 ml of blood was collected from each animal and, after gently mixing, kept in a cooler box. The blood was transferred to the laboratory and processed within eight hours of collection.

### Dispensing of blood

A 24-well tissue culture plate was used to divide the blood sample from each animal into four aliquots (**Figure 4**). The rows of the plate were marked B in the first row for bovine antigen, A in the second row for avian antigen, F in the third row for fortuitum PPD antigen and C in the fourth row for the control. Aliquots of 1.5 ml blood from each animal were dispensed into each well labeled B, A, F and C using sterile disposable pipettes.



**Figure 4** Layout of the 24-well tissue culture plate

### Stimulation of blood with Mycobacterial antigens

In all the wells labeled B, 30  $\mu$ l of bovine tuberculin was added. In all the wells labeled A, 60  $\mu$ l of avian tuberculin was added. In all the wells labeled F, 25  $\mu$ l of fortuitum PPD was added. The wells labeled C were the controls and contained only the test samples. The antigens and blood were gently mixed in a micro-plate shaker.

## 2.4.2 Detection of Interferon gamma by Enzyme Immune Assay

### Incubation of plates

After slight shaking, all the plates were incubated at 37 °C for 24 hours.

### Harvesting of plasma

For each animal, four micro-centrifuge tubes were labeled with the animal's identity and then B, A, F and C respectively. Plasma was harvested from corresponding wells and placed into respective micro-centrifuge tubes. The sera were stored in a freezer until processed.

### Bovine interferon gamma-Enzyme Immune Assay

- A. All plates and reagents, except for the conjugate, were removed from the storage fridge/freezer at least one hour before the test to bring them to room temperature.
- B. 96 well plates were used, testing 92 samples at a time. Plates were labeled with the date on which the test was done, the serial number of the plate, the corresponding identity of each blood sample in the well and the negative and positive controls.
- C. 50 µl of green diluent was added into each well. For each animal, 50 µl of the B-plasma was added into each well with the green diluent. Two negative samples and two positive samples were added into the last four wells of each plate. Mixing was achieved by pipetting up and down three times after each addition of the plasma to the green diluent in the well.

The plate was covered with a lid and then incubated at room temperature on a plate shaker at 600 rpm for one hour.

- D. After incubation, wells were washed six times using a wash buffer as recommended by the manufacturer. After the last wash, plates were placed facing down on a filter paper, with occasional flicking, to allow for drainage.
- E. 100 µl of freshly prepared conjugate, as recommended by the manufacture, was added into each well and mixed by pipetting up and down three times. The plates were then incubated at room temperature on a plate shaker at 600 rpm for one hour.
- F. After incubation, the plates were washed as before.
- G. 100 µl of freshly prepared enzyme substrate solution was added into the wells and mixed by pipetting up and down three times. Again, the plates were covered and incubated for 30 minutes away from direct sunlight.
- H. After this incubation, 50 µl of Enzyme stopping solution is added into all wells. The plates were then read at 450 nm filter with a 650 nm reference filter and absorbance values obtained.

- I. Before the absorbance for each sample was noted, the absorbance of the negative and positive controls was observed. Absorbance of samples was only accepted if the negative controls were test negative, i.e. negative control  $< 0.20$ , and the positive controls were test positive, i.e. positive control  $> 1.0$ .
- J. All samples whose OD reading was less than 0.38 were considered negative and no further testing were done on them.

All bovine PPD stimulated plasma samples that yielded an OD reading of above 0.38 were considered as suspect BTB cases and were retested.

Retesting of suspect cases

- K. Plates were labeled as before. In addition, for each test samples, the A, F and C plasma were added in addition to the B plasma. Negative and positive controls were added as before.
- L. The entire IF-gamma assay was repeated as described above, up to the reading of the absorbance

### **Interpretation of absorbance (OD) readings**

Note: as stated above, the positive test kit control should be  $> 1.0$  and the negative control should be  $< 0.20$  before the OD readings of a plate could be accepted.

1. If OD-nil  $> 0.35$  : result is invalid
2. If OD-bov below 0.38 - the test result is negative for BTB. No need for further testing.
3. If     i) OD-bov - OD-av  $> 0.20$   
      and ii) OD-nil 0.35  
      and iii) OD-fort - OD-nil  $\leq 0.15$

the test result of this animal is positive for BTB.

Other possible test outcomes:

4. If OD-bov - OD-av  $< 0.20$  but  $> 0$ , then negative for *M. bovis*
5. If OD-av  $> OD-bov$ , then animal is an avian reactor
6. If OD-fort - OD-nil  $\geq 0.15$  and OD-bov - OD-av  $> 20$ , then animal is a multiple reactor

## **2.5 Bovigam 2G (IFN- $\gamma$ ) Assay**

The Bovigam B2G test is a modified and improved version of the Bovigam 1G test. Cattle were tested using the Bovigam 2G test according to the manufacturer's instructions (Prionics,

Switzerland) ([www.prionics.com](http://www.prionics.com)). This test was not part of the objective but a window of opportunity was provided when Prionics sponsored a limited number of B2G test kits.

### **2.5.1 Whole blood culture**

#### **Blood collection**

Blood collecting tubes containing heparin as an anti-coagulant were clearly marked with the identity of each animal. Each animal was bled by veno-puncture of the jugular vein using 18G vacutainer needles whereby 2-3 ml of blood was collected from each animal and, after gently mixing, kept in a cooler box. The blood was transferred to the laboratory and processed within eight hours of collection.

#### **Dispensing blood**

250  $\mu$ l of aliquots of heparinised blood from each animal was dispensed into wells of a 96-well culture tray. Blood of each animal is dispensed into three wells: NL - Nil control antigen, aP - avian PPD and aB - bovine PPD.

#### **Addition of stimulation antigens**

25  $\mu$ l of PBS was added to each NL, 25  $\mu$ l of avian PPD was added to each aP well and 25  $\mu$ l of bovine PPD was added to each aB well in the culture tray. The antigens were mixed thoroughly into the aliquoted blood.

#### **Incubation**

Culture trays were incubated at 37 °C for 24 hours in a humidified atmosphere.

#### **Harvesting plasma**

100  $\mu$ l of plasma was harvested from each well and transferred to storage tubes and frozen.

### **2.5.2 Bovine IFN- $\gamma$ EIA**

- A. Freeze dried components were reconstituted while other reagents were equilibrated.
- B. 50  $\mu$ l of green diluents was added to each well of a 96-plate culture tray.

- C. 50 µl of sample plasma was added to the wells. 50 µl of control sample was added to respective control wells. Mixing was achieved by shaking for one minute in a microplate shaker.
- D. Culture plate was covered with a lid and incubated at room temperature on a plate shaker set at 600 rpm for one hour.
- E. Contents were shaken out and plate was washed four times with a wash buffer.
- F. 100 µl of freshly prepared conjugate reagent was added to the wells.
- G. Culture tray was then covered with a lid and incubated at room temperature as in D above.
- H. Wells were washed as in E above.
- I. 100 µl of enzyme substrate was added to wells followed by mixing.
- J. Culture tray was incubated on a microplate shaker for 30 minutes, away from direct sunlight.
- K. 50 µl Enzyme stopping solution was added to each well.
- L. Absorbance of each well was read within five minutes of terminating reaction using a 450 nm filter with a 620-650 nm reference filter.

### **Interpretation of OD readings**

The OD readings of the nil antigen, avian and bovine PPD are compared.

Positive = OD bovine PPD - Nil antigen  $\geq$  0.1, and OD bovine PPD - OD avian PPD  $\geq$  0.1

Negative = OD bovine PPD - Nil antigen  $<$  0.1, and OD bovine PPD - avian PPD  $<$  0.1.

## **2.6 Conducting questionnaire survey**

### **2.6.1 Designing questionnaire and conducting interviews**

A questionnaire was carefully designed to study the zoonotic aspects of BTB and determine the risk faced by farmers and consumers of cattle products such as milk and meat. The questionnaire prompted farmers to respond to questions pertaining the management and health status of their herds, with particular emphasis to BTB. It also induced farmers to respond to production and consumption practices of products of cattle origin.

The questionnaire was designed in the English Language and then translated into the SiSwati language. The SiSwati version was translated back to the English language and the two English versions compared for similarities and differences. Any loss in translation, such as

ambiguous or dubious phrases, was corrected and a final English and SiSwati version was used.

### **Training survey assistants**

In addition to the researcher, two assistants were trained to conduct the survey. They were familiarized with the questionnaire, and each question was explained and interpreted to them. A simulation exercise was also conducted, where the assistants interviewed mock farmers.

### **Conducting interviews**

Interviews were conducted with farmers concurrently with the comparative intra-dermal skin test. Farmers were interviewed in the language of their choice, but a majority of the farmers preferred an interview in both languages, drifting from one language to another to ensure that they understand what was being asked from them. Before the interview was conducted, the farmer was assured of the confidentiality of the information to be provided by him/her and no names/kraal numbers were required from the farmers.

## **2.7 Analysing Data**

### **General**

Statistical Package for the Social Sciences (SPSS) Version 20 (IBM Statistics) was used for the descriptive analysis and to determine whether there was an association of BTB with gender, region, herds size group and dip tank type, and for calculating Kappa. All calculations were computer based. For the descriptive analysis, an Excel data base was generated containing all the results obtained from CIST and the IFN- $\gamma$  tests and the responses obtained from the questionnaire survey.

### **BTB Association with Gender, Region, Dip tank type and Herd size group**

For the determination of the association of BTB with gender, region, herd size and dip tank type, a logistic regression model was used. Logistic regression is a form of regression that uses logic transformation to calculate the ratio of probability by using probability outcome divided by probability without it and to predict the probabilities of group memberships in relation to several variables (Jinga *et al.*, 2013). It calculates the probability of an event to occur (Jinga *et al.*, 2013). The dichotomous dependant variables that was used was BTB and independent

variables were gender, dip tank type, region and herd size group. All variables in the model were categorical and were:

### Dependant Variable

BTB Status: BTB positive and BTB negative

### Independent Variables

- A. Sex: male and female
- B. Dip Tank type: Swazi Nation Land and Private Farms
- C. Region: Hhohho, Manzini, Shiselweni and Lubombo
- D. Herd size: Below 80 cattle and 80 plus cattle. The basis for 80 heads of cattle to be the cut-off point was based on the observed fact that in the majority of dip tanks in the Swazi Nation Land, they were very few herds which had more than 80 heads of cattle, while the opposite was true for dip tanks on Private Farms. When the herd size group was plotted on a graph, there was a distinct change on the gradient curve at 80 heads of cattle.

The categorical variables were modeled as dummy variables in the analysis. Dummy variables are numerical variables that usually represents a binary categorical variable such as sex, dip tank type, location, etc., which enables the use of a single regression equation for categorical variables and act like switches that turn various parameters on and off in an equation (Xiong *et al.*, 2006).

For every  $k$  categories,  $k-1$  categories were included in the model, as follows:

- A. Sex: one category (female) used, male is a reference category
- B. Dip Tank type: One category (SNL) used, private is a reference category.
- C. Region: Three categories used (Hhohho, Manzini, Shiselweni) used, Lubombo is a reference category.
- D. Dip Tank type: One category used (Below 80 cattle), 80 plus cattle used as a reference category.

Thus the logistic regression model is represented as,

$$Y_i = \beta_0 + \beta_1 \text{male} + \beta_2 80 \text{ plus cattle} + \beta_3 \text{Hhohho} + \beta_4 \text{Manzini} + \beta_5 \text{Shiselweni} + \beta_6 \text{Private} + \varepsilon_i$$

Where  $Y_i$  = Presence, and non-presence of BTB.

$\beta_0$  = intercept

$\beta_i$  = regression coefficients

## Calculating Kappa

Cohen's Kappa (Warriers, 2011) was calculated using the following equation or formula:

$$K = \frac{Pr(a) - Pr(e)}{1 - Pr(e)}$$

Where K = Cohen's Kappa

Pr(a) = the observed agreement

Pr(e) = the hypothetical probability of chance agreement

## Calculating True Prevalence

The true prevalence (Liapi *et al.*, 2011) of BTB in the cattle population was calculated using the following formula:

$$TP = (P + SP - 1) / (SE + SP - 1)$$

Where TP = True prevalence, P = Prevalence, SP = Specificity and SE = Sensitivity

## Questionnaire survey

For the questionnaire survey, responses for each of the questions were recorded in the Excel database.

Responses to the questionnaire were entered as number codes. Further analysis was made by calculating frequencies and/or percentages per dip tank.

## Chapter 3

### Results

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#### 3.1 Comparative Intra-dermal Skin Test (CIST)

In all the dip tanks of study, 1 485 heads of cattle were inoculated with bovine and avian PPD. These included 438 male animals and 1 047 female animals. However, only 1 333 heads of cattle, consisting of 396 males and 937 females, were returned by their owners for reading of the CIST and their BTB results were recorded (**Table 4**).

The 1 475 heads of cattle that were inoculated belonged to 504 herds while the 1 333 heads of cattle which were read belonged to 442 herds. The absent animals belonged to 62 herds.

The 1 333 heads of cattle represented 10.3 % of the cattle in the dip tanks of study. Of the males and females inoculated, 9.8 % and 9.9 % respectively were absent on the days when the test was to be read.

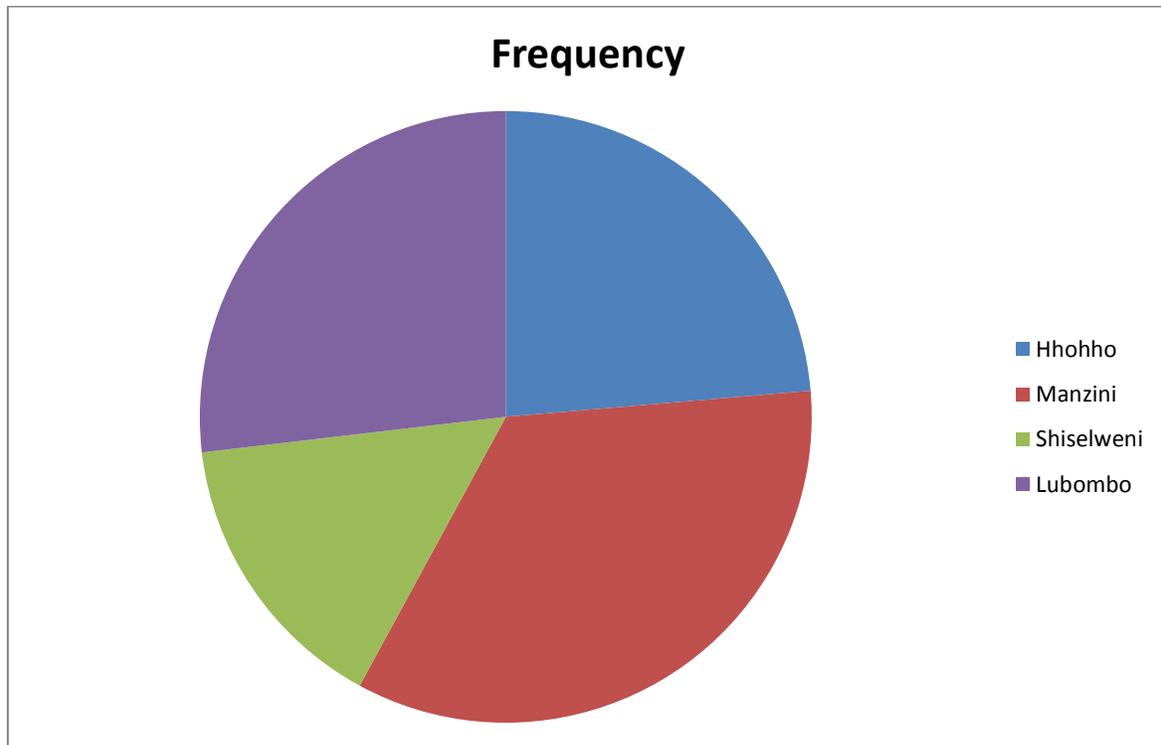
The distribution of cattle which completed BTB testing per district and sex is illustrated in **Table 3** and **Figure 5** and shows that approximately one third of cattle were tested in the Manzini district and comprised 70 % female animals and 30 % male animals.

**Table 4** Regional and sex distribution of cattle tested for BTB

	District	Frequency	Percentage	Valid %	Cumulative %
Valid	Hhohho	315	23.6	23.6	23.6
	Manzini	457	34.3	34.3	57.9
	Shiselweni	203	15.2	15.2	73.1
	Lubombo	358	26.9	26.9	100
<b>Total</b>		<b>1 333</b>	<b>100</b>	<b>100</b>	

	Sex	Frequency	Percentage	Valid %	Cumulative %
Valid	Male	393	29.5	29.5	29.5
	Female	940	70.5	70.5	100
<b>Total</b>		<b>1333</b>	<b>100</b>	<b>100</b>	



**Figure 5** Frequency Pie Chart showing the number of cattle tested per region

From visual inspection and palpation of the injection sites of the avian and bovine, a number of qualitative observations were made in different animals, apart from those arising from poor shaving techniques. These observations appeared in isolation and sometimes in combination and included necrosis, enlargement and inflammation of the injection sites, oedema, exudates and pain (**Table 5**). In some cattle, such as those at EIEI-Corporation, the enlargement at the bovine injection sites persisted and was still visible up to four weeks after inoculation of bovine PPD.

**Table 5** Qualitative observations associated with the skin test

Lesion	Avian	Bovine
Necrosis	1	0
Inflammation	60	135
Oedema	59	98
Enlargement of injection site	47	137
Exudation	0	1
Pain	0	135

Using a bovine bias equal to or greater than 4 mm, all the 16 dip tanks of study were found to have at least one animal positive for BTB and several animals with inconclusive results. There were 90 cattle that tested positive for BTB, consisting of 28 males and 62 females. These cattle were in 48 different herds. For 65 cattle, made up of 22 males and 43 females, the results for the CIST were inconclusive. These cattle were in 44 different herds. A total of 1 177 cattle, consisting of 445 males and 848 females, and belonging to 349 different herds, were found to be negative for BTB (**Table 6**).

The prevalence of BTB in the study population was found to be 6.75 %. The prevalence of BTB in male cattle was 7.07 % while it was slightly less in female cattle at 6.62 %. Of the 441 herds of cattle that were tested, 10.88 % were positive for BTB (**Table 6**). Just over 88 % of the cattle tested were negative for BTB, reflecting about 79 % of all herds tested. These animals had a bovine bias of less than 2 mm. Of all the male and female animals tested, 87.34 % and 88.98 % were negative, respectively (**Table 6**).

The animals that were designated as inconclusive were those that, based on the CIST test performed, could neither be classified as positive nor negative and had a bovine bias between 2 mm and 4 mm. The inconclusive made up of 4.88 % of all animals tested and belonged to 9.98 % of the herds tested. Of all the males and females tested, 5.56 % and 4.59 % were inconclusive, respectively (**Table 6**).

**Table 6** Summary of the results of the comparative intra-dermal skin test in 16 dip tanks

	Study population	Tested (%)	Positive	%	Negative	%	Inconclusive	%
Total number of animals tested	1 293	1 333 (10.3)	90	6.75	1 178	88.3	65	4.88
Number of herds tested	504	441 (87.5)	48	10.88	349	79.14	44	9.98
Total number of male animals tested	-	396	28	7.07	346	87.37	22	5.56
Total number of female animals tested	-	937	62	6.62	832	88.79	43	4.59

BTB was found to be present in all 16 dip tanks of study where the prevalence was variable, ranging from 0.59 in Mpofo Dip tank to 27.66 in EIEI-Corporation (**Table 7**). Two dip tanks which were located in privately owned farms, EIEI-Corporation and TM Corporation, had the highest BTB prevalence at 27.27 and 27.66 % respectively, while Mpofo had the lowest BTB prevalence at 0.59 % (**Table 7**). The rest of the dip tanks of study had a prevalence ranging from 3.09 % - 9.7 %.

In terms of individual animals, EIEI-Corporation had the highest number of animals testing positive for BTB at 26 followed by Croydon and TM-Coop at 10 and nine animals, respectively. A majority of the dip tanks of study had either three or four animals positive for BTB, while Mpofo dip tank had only one animal positive for BTB (**Table 7**).

Piennarr dip tank, followed by EIEI-Corporation had the highest proportion of inconclusive animals at 9.35 % and 9.57 %, respectively, while Mafutheni had the least, excluding TM-Cooperation which had none, at 1.9 %.

**Table 7** BTB prevalence in the 16 dip tanks of study

Dip Tank	No of cattle	Tested	%	Positive	%	Inconclusive	%	Negative	%
Croydon	1 594	103	6.46	10	9.7	4	3.88	89	86.41
Langkraal	379	43	11.35	3	6.98	2	4.65	88	88.37
TM-Coop	164	33	20.12	9	27.27	0	0	24	72.73
EIE-Coop	301	94	31.23	26	27.66	9	9.57	89	62.77
Matata	432	47	10.88	3	6.38	3	6.38	41	87.23
Thunzini	883	98	11.09	4	4.08	2	2.04	92	93.88
Piennarr	1 164	107	9.19	7	6.54	10	9.35	90	84.11
Luphala	874	97	11.09	3	3.09	5	5.16	89	91.75
Mkhiwa	1 057	91	8.61	3	3.29	5	5.49	83	91.21
Mtilane	1 024	79	7.71	4	5.06	4	5.06	71	89.87
Sigombeni	1 258	96	7.63	4	4.17	1	1.04	91	94.79
Elukwatini	954	64	6.71	4	6.25	3	4.69	57	89.06
Metulo	1 017	82	8.06	3	3.66	4	4.88	74	90.24
Lovunga	781	78	9.99	3	3.85	5	6.41	70	89.74
Mpofu	875	169	19.31	1	0.59	7	4.14	161	95.27
Mafutheni	236	52	22.03	3	5.77	1	1.9	48	92.31
<b>TOTAL</b>	<b>12 993</b>	<b>1 333</b>	<b>10.26</b>	<b>90</b>	<b>6.75</b>	<b>65</b>	<b>4.88</b>	<b>1 177</b>	<b>88.29</b>

For herds where more than 15 cattle were tested for BTB, the prevalence of BTB in each herd was calculated (**Table 8**). Out of these 11 herds, only one herd, Mpofu (I) was found to have a BTB prevalence of zero while the rest had BTB prevalence ranging from 1.25 % - 27.66 %.

**Table 8** Intra-herd BTB prevalence in selected herds

Dip tank	Herd number	Tested	Positive	%	Inconclusive	%	Negative	%
EIE-Cop	595	94	26	27.66	9	9.56	59	62.77
TM-Cop	2 013	33	9	27.27	0	0	24	72.73
Mpofu	197	80	1	1.25	3	3.75	76	95
Mpofu	1	81	0	0	4	4.94	77	95.06
Lovunga	197	78	3	3.84	5	6.41	70	89.74
Thunzini	273	49	3	6.12	3	6.122	43	87.75
Thunzini	655	49	1	2.04	1	2.04	47	95.91
Mafutheni	F	34	2	5.88	0	0	32	94.11
Mafutheni	V	17	1	5.88	2	11.76	14	82.35
Matata	31	47	3	6.38	3	6.38	41	87.23
Langkraal	111	43	3	6.98	2	4.65	38	88.37

The test negative animals were further categorized into the true negatives, those with a bovine bias of  $0 \leq x \leq 2$  and the avian reactors, those with a bovine bias of  $x \leq 0$ , as shown in **Table 9**.

**Table 9** Test results of the intradermal tuberculin test in 1 333 animals

	<b>Tested</b>	<b>Avian Reactors (%)</b>	<b>True Negatives</b>	<b>Percentage</b>	<b>Positive ≥ 4 mm</b>	<b>Percentage</b>	<b>Negative ≥ 4 mm</b>	<b>Percentage</b>	<b>Inconclusive ≥ 4 mm (%)</b>
Number of animals	1 333 (10.3 %)	652 (48.9 %)	525	39.38	90	6.75	1 177	88.3	65 (4.88 %)
Number of herds	441 (87.5 %)	233 (52.83 %)	116	26.3	48	10.88	349	79.14	44 (9.98 %)
Number of males	395 (43 absent)	174 (44.05 %)	172	43.54	28	7.09	346	87.59	22 (5.7 %)
Number of females	953 (99 absent)	565 (59.28 %)	283		62 (6.51 %)		848 (88.98 %)		43 (4.51 %)

## True Prevalence

At the cut-off point for test positive animals of  $\geq 4$  mm, the true prevalence was calculated as follows:

$$\begin{aligned} TP &= (P + SP - 1) / (SE + SP - 1) \\ P &= 6.75 \\ SP &= 97 \% = 97 / 100 = 0.97 \\ SE &= 59 \% = 59 / 100 = 0.59 \\ \text{Confidence interval} &= 95 \% \\ &= (6.75 + 0.97 - 1) / (0.59 + 0.97 - 1) \\ &= 12 \\ TP &= 12 \end{aligned}$$

## 3.2 Results for Culture

From EIEI-Corporation, seven heads of cattle that had tested positive for BTB during the comparative intra-dermal skin test were slaughtered, at the Swaziland Meat Industries' abattoir while one BTB test positive animal from Lovunga was slaughtered at its kraal. A *post mortem* examination was performed and all the eight animals had tuberculous lesions. Samples were collected from these lesions and cultured at the DVTD BSL2+ Laboratory.

Of these eight cattle slaughtered, *M. bovis* was isolated from seven animals from EIEI-Cooperation, but could not be isolated from tissues of the BTB test positive cattle from Lovunga dip tank (**Table 10**).

**Table 10** Isolation of *M. bovis* from samples obtained from slaughtered BTB positive cattle

Dip Tank	Animal ID	Laboratory ID	Culture for <i>M. bovis</i>
EIE-CORP	9	65752	Positive
EIE-CORP	28	65753	Positive
EIE-CORP	31	65754	Positive
EIE-CORP	8	65764	Positive
EIE-CORP	39	65769	Positive
EIE-CORP	116	65766	Positive
LOVUNGA	GUGU	Lovunga CX3	Negative

### 3.3 Results for IFN- $\gamma$ (Bovigam 1G) Test

The IFN- $\gamma$  (Bovigam 1G) Test was performed on bovine blood samples collected in seven dip tanks, namely Elukwatini, Metulo, Lovunga, Mpofu and EIEI-Corporation, Croydon and TM-Corporation, in parallel with CIST. In total, 581 animals from 173 herds were tested for BTB using the IFN- $\gamma$  (1G) Test. While all these animals had been enrolled for BTB testing using CIST, 32 animals did not complete the CIST and hence their CIST status was unknown. Four of these animals were positive for BTB while 28 were negative for BTB when tested using the IFN- $\gamma$  (1G) Test (**Table 11**).

Of the 581 animals tested, 26 were positive for BTB in the IFN- $\gamma$  (1G) test, resulting in a prevalence of 4.48 %. Of these 26 animals, seven were males while 19 were females, giving respective prevalence of 5.69 % and 4.15 %. Of the 173 herds that were tested, 14 were positive for BTB, resulting in a herd prevalence of 6.67 % (**Table 11**).

Eight of the animals had results that were invalid and these constituted one male and seven females all coming from six herds. Thirty-three animals were avian reactors and they constituted of 30 females and three males, from eight herds. Three female animals coming from two herds were multiple reactors. There were 511 animals that tested negative for BTB and these included 112 males and 399 females coming from 143 herds. The percentage of negative animals was 87.95 % of the total animals tested with the IFN- $\gamma$  (1G) Test, with 91.06 % males and 87.12 % females tested being negative, while 81.91 of all herds tested were negative (**Table 11**).

**Table 11** Summary of the IFN- $\gamma$  (1G) Test Results

	Total	IFN- $\gamma$ +ve	Invalid	Avian	Multip	IFN $\gamma$ -ve
Animals tested	581	26 (4.48 %)	8 (1.38 %)	33 (5.68 %)	3 (0.52 %)	511 (87.95 %)
Herds tested	173	14 (6.67 %)	N/A	N/A	N/A	143 (82.85 %)
Males tested	123	7 (5.69 %)	1 (0.81 %)	3 (2.44 %)	0 (0 %)	112 (91.06 %)
Females tested	458	19 (4.15 %)	7 (1.53 %)	30 (6.55 %)	3 (0.66 %)	399 (87.12 %)

+ve-Test Positive  
 Avian-Avian Reactor  
 Multip-Multiple Reactor  
 -ve-Test Negative

As with CIST, at least one animal was found to be positive for BTB per dip tank in all dip tanks that were tested with the IFN- $\gamma$  Test, with the exception of TM-Corporation. As with CIST, the prevalence of BTB was found to be highest at EIEI-Corporation at 16.18 % while it was lowest at Lovunga, at 1.12 %. All dip tanks with the exception of Lovunga identified animals with invalid results, which were 2.28 % of all the animals tested. Avian reactors were detected in all the dip tanks, except for Elukwatini. These constituted 3.33 % of all animals tested. One multiple-reactor was found at EIEI-Corporation (**Table 12**).

**Table 12** IFN- $\gamma$  (Bovigam 1G) Test Results for Selected Dip Tanks

Dip tank	Total No of Cattle	Tested	%	+ve	%	Invalid	%	Avian	%	Multip	%	-ve	%
Elukwatini	954	64	6.71	2	3.1	1	1.56	0	0	0	0	61	95.31
Metulo	1 017	87	8.55	2	2.29	3	3.45	2	2.29	0	0	80	91.95
EI-Corp	301	68	22.59	11	16.18	5	7.35	6	8.82	1	1.47	45	66.18
Lovunga	781	89	11.39	1	1.12	0	0	7	7.87	0	0	78	87.64
Mpofu	875	89	10.17	3	3.37	2	2.25	1	1.12	0	0	82	92.13
Crydon	1 594	113	7.09	7	6.19	1	0.88	1	0.88	0	0	104	92.04
TM-Corp	164	61	37.19	0	0	1	1.64	2	3.28	0	0	58	95.08
Total	5 686	571	10.04	26	4.55	13	2.28	19	3.33	1	0.18	508	88.96

+ve-Test Positive  
 Avian-Avian Reactor  
 Multip-Multiple reactor  
 -ve-Test Negative

### 3.4 Comparison between CIST and IFN- $\gamma$ (1G) Results

In the seven dip tanks listed in **Table 12** above, a total of 623 heads of cattle were tested for BTB with CIST while 581 were tested with IFN- $\gamma$  (1G). The objective was to test the sampled cattle in these dip tanks with both tests but due to logistical constraints and the fact that skin test and IFN- $\gamma$  (1G) were not performed on the same day completely synchronized, this was not possible in all cases. Another reason was the non-compliance of farmers to return injected cattle that had already been bled for the IFN- $\gamma$  (1G) Test, meaning that they could not complete the skin test and hence their results for the skin test was unknown. A further reason was failure to bleed animals that had been tested with the skin test, meaning their IFN- $\gamma$  (1G) test could not be done.

In these seven dip tanks, 82 heads of cattle were identified as positive by either CIST or IFN- $\gamma$  (1G) or both tests. These 82 heads of cattle included eight animals that were identified by both tests to be positive for BTB. CIST identified 56 animals as positive for BTB and these included the eight that were also identified as positive by the IFN- $\gamma$  (1G), 28 animals that tested negative, one animal with an invalid result, three that were avian reactor, one that was a multiple reactor and 15 animals that were not tested by the IFN- $\gamma$ (1G). The IFN- $\gamma$  (1G) test identified 26 animals as positive for BTB and this included the eight animals that were positive in CIST, 10 animals that were negative in CIST, one inconclusive animal and seven animals that were not tested by CIST.

However, 452 animals were tested for BTB by both CIST and IFN- $\gamma$  (1G) in these seven dip tanks (**Table 13**). CIST identified 41 animals as positive for BTB while 392 were negative and 19 had inconclusive results (**Table 13**). The IFN- $\gamma$  (1G) assay identified 21 animals as positive while 13 had invalid results, 16 were avian reactors and one was a multiple reactor (**Table 13**). Again, eight animals were identified as positive by both tests (**Table 13**).

**Table 13** A comparison between CIST and IFN- $\gamma$  (1G) Assay in the 452 animals that were tested with both tests

	CIST positive	CIST negative	Total
IFN- $\gamma$ 1G positive	8	13	21
IFN- $\gamma$ 1G negative	33	398	431
<b>Total</b>	<b>41</b>	<b>411</b>	<b>452</b>

The observed agreement between CIST and IFN- $\gamma$  1G is 0.89 and the expected agreement is 0.87. Kappa ( $\kappa$ ), therefore, is calculated to be 0.209. There is a low or slight agreement between the CIST and the IFN- $\gamma$  tests. The positive agreement was 0.26 and the negative agreement was 0.95. The positive agreement means that in this study, CIST and IFN- $\gamma$  identified the same test positive animals in 26 % of the animals while the negative agreement means that the two tests identified the same test negative animals in 95 % of cases.

### 3.5 Results for the Second Generation IFN- $\gamma$ (Bovigam 2G) Assay

In two dip tanks, Croydon and TM-Corporation, 91 heads of cattle were tested with the second generation IFN- $\gamma$  (Bovigam 2G) Assay (**Table 14**). These animals were from 58 herds and included 27 males and 65 females. Only three heads of cattle, coming from two herd animals tested positive with Bovigam 2 and these constituted two males and one female. The rest of the animals tested negative for BTB when tested using IFN- $\gamma$  (2G) (**Table 14**).

**Table 14** Results for the IFN- $\gamma$  2G test

	<b>Tested</b>	<b>Test Positive</b>	<b>Test Negative</b>
Total number of animals tested	91	3 (3.29 %)	88 (96.74 %)
Number of herds	58	2 (3.45 %)	56 (96.55 %)
Males	27	2 (7.4 %)	25 (92.59 %)
Females	65	1 (1.54 %)	64 (98.46 %)

### 3.6 Comparison between of the IFN- $\gamma$ (1G) and IFN- $\gamma$ (2G) Assays

The IFN- $\gamma$  (1G) and IFN- $\gamma$  (2G) tests were performed on 89 heads of cattle at Croydon and TM-Corporation dip tank (**Table 15**). In all, 89 heads of cattle were tested for BTB using the IFN- $\gamma$  (1G) and IFN- $\gamma$  (2G) tests. Seven heads of cattle tested positive in the IFN- $\gamma$  (1G) test and three heads of cattle tested positive in the IFN- $\gamma$  (2G) test (**Table 15**). Two heads of cattle tested positive in both tests.

**Table 15** Comparison of IFN- $\gamma$  (1G) and IFN- $\gamma$  (2G) assays

	IFN- $\gamma$ (1G) Positive	IFN- $\gamma$ (1G) Negative	Total
IFN- $\gamma$ (2G) Positive	2	1	3
IFN- $\gamma$ (2G) Negative	5	81	86
<b>Total</b>	<b>7</b>	<b>82</b>	<b>89</b>

The observed agreement is 0.93 and the expected agreement is 0.089. Therefore, Kappa ( $\kappa$ ), is calculated to be 0.370. There is almost perfect agreement between the IFN- $\gamma$  (1G) and IFN- $\gamma$  (2G) tests. The positive agreement for the two tests was 0.26 (or 26 %) while the negative agreement was 0.95 (or 95 %). This means that the two tests identified test positive and test negative animals 26 % and 95 % of the time respectively.

### 3.7 BTB's association with gender, region, relative herd size and dip tank type of the animal

According to **Table 16**, the logistics analysis results gives the P-values, sex of an animal (male), is 0.383, Dip tank type (Private) is 0.169, herd size group (80 plus cattle) is 0.950. The P-values for the regions are: (Hhohho) is 0.33, (Manzini) is 0.0005, (Shiselweni) is 0.133 (**Table 16**). The odds for the sex of an animal (male), Dink tank type (Private) and herd size group are 1.227, 0.436 and 0.961 respectively. The odds for the regions are 0.646, 4.101 and 2.066 for the Hhohho, Manzini and Shiselweni regions respectively (**Table 16**).

**Table 16** Regression analysis

Variable		Frequency	df	Co-efficients	Odds	P-Value
Region	Hhohho	315	1	-0.437	0.646	0.330
	Manzini	457	1	1.411	4.101	0.0005
	Shiselweni	203	1	0.726	2.066	0.133
	Lubombo	358	-	-	-	-
Herd Size group	Less than 80 cattle	646	-	-	-	-
	80 plus cattle	687	1	-0.40	0.961	0.961
Dip Tank Type	Swazi Nation Land	714	-	-	-	-
	Private	619	1	-0.831	0.436	0.436
Sex	Male	396	-	-	-	-
	Female	937	1	0.205	1.227	1.227

### 3.8 Questionnaire Survey

#### 3.8.1 Herd demographics

Cattle numbers in the various kraals in the dip tanks of study were variable, ranging from four to 256 heads of cattle, with 73.9 % of kraals comprising 10-30 heads of cattle (**Table 17**).

**Table 17** Relative number of cattle per kraal in the dip tanks of study

Number of cattle per kraal	Number of farmers	Percentage
1-9	146	18.5
10-30	585	73.9
≥ 30	58	7.9
<b>Total</b>	<b>789</b>	<b>100</b>

Forty or 5.19 % of the farmers interviewed reported that their animals had calves while 16 farmers or 2.02 % indicated that their animals were pregnant. In all the dip tanks studied, 38.97 % of farmers indicated that they had at least one head of cattle that was six years old and above.

Eighty-eight or 11.1 % of the farmers interviewed reported that their animals were sick or undergoing treatment (**Table 18**), with 4.2 % of them already receiving treatment. In 5.8 % of the cases, the farmers stated that the animals have been sick for a prolonged period (**Table 18**). While 2.4 % of the farmers stated that their cattle died on their own, 5.3 % claimed that their cattle died because of their sickness while 7.7 % of the farmers stated that they killed their cattle rather than watch them die of their sickness (**Table 18**).

**Table 18** Health status of cattle as reported by farmers

	Number of responses	Percentage
Presence of sick cattle in kraal	88	11.1
Cattle showing prolonged sickness	46	5.8
Cattle showing emaciation	58	7.3
Cattle lagging behind others	11	1.4
Cattle that died on their own	19	2.4
Sick cattle that eventually died	42	5.3
Cattle slaughtered due to sickness	61	7.7
Cattle currently receiving treatment	33	4.2
Sick cattle that recovered	53	6.7

### 3.8.2 Cattle Management

Management of cattle was basic, with grazing being the principal source of feed for the animals. Only 1.4 % and 0.5 % of the farmers interviewed provided their cattle with extra feed and water in the kraals, respectively. All cattle are kept at their respective kraals overnight and none of the farmers provided internal housing for their animals. Disease diagnosis for sick animal is mainly based on clinical signs observed by the farmer and, later, the veterinary assistant responsible for that particular dip tank. There is no laboratory or any other investigative diagnosis except in a few cases, such as in cattle with perpetual abortions. The veterinary assistant, who is the animal health technician responsible for each dip tank, is responsible for attending to sick animals when consulted, including prescribing and, sometimes, administering, medication. Up to 93.9 % of the farmers interviewed exclusively consult the veterinary assistant, with the latter being responsible for referring the farmer to a veterinarian or directly consulting the veterinarian on behalf of the farmer. For assistance with sick animals, 13.4 % of the farmers consulted a veterinary surgeon on a regular basis. At 33.3 %, a third of the farmers administer drugs for the

control of internal parasites on a regular basis to their cattle, with the rest giving them sporadically or none at all. Only 2.4 % of the farmers administer to their cattle drugs for the control of external parasites, with the rest relying solely on the acaricides freely provided by the government at the dip tank (**Table 19**).

**Table 19** General cattle management in the dip tanks of study

Management practice	Yes		No	
	No of farmers	Percentage	No of farmers	Percentage
Provision of extra feed	11	1.4	778	98.6
Provision of water in kraals	4	0.5	784	99.5
Provision of salt licks	56	7.1	733	92.9
Regular control of internal parasites	264	33.5	525	67
Regular control of external parasites	19	2.4	770	97.6
Veterinary consultations	17	2.1	772	86.6
Consultation of animal health technicians	741	93.9	48	6.1
Housing for calves	0	0	789	100
Housing for adult animals	0	0	789	100
Grazing as a major source of feed	789	100	0	0

### 3.8.3 Cattle movement patterns

Of the farmers interviewed, 17.8 % had permitted in (received) new animals into their kraal while 18.8 % of the farmers had permitted out animals from their kraals in the last six months preceding the study. Of the former group of farmers, 22.9 % permitted in cattle from the same dip tank, 39.7 % permitted in cattle from a dip tank in the same sub region, 32.6 % permitted in cattle from a dip tank in the same region and 17 % permitted in cattle from a dip tank in other regions of the country. Of the latter group of farmers, 20.1 % permitted cattle out to other kraals in the same dip tank, 44.9 % permitted cattle out to dip tanks in the same sub region while 29.5 % permitted cattle out to dip tanks in the same region while the rest, being 9.2 % permitted cattle out to other regions of the country. None of the cattle received or sent away were clinically examined or tested for the presence of BTB (**Table 20**).

**Table 20** Cattle movements within and between the dip tanks of study and other areas

Farmers permitting cattle in				Farmers permitting cattle out			
Origin of cattle	No of farmers	% farmers	% interviewed	Destination of cattle	No of farmers	% farmers	% interviewed
Local dip tank	31	20.1	3.9	Local dip tank	30	20.1	3.8
Sub-regional dip tank	56	37.6	7.1	Sub-regional dip tank	67	44.9	8.5
Regional dip tank	46	30.9	5.8	Regional dip tank	44	29.5	5.6
Dip tank in other regions	9	11.4	2.0	Dip tank in other regions	8	5.5	1.0
<b>Total</b>	<b>141</b>	<b>100</b>	<b>18.8</b>	<b>Total</b>	<b>141</b>	<b>100</b>	<b>18.0</b>

In total, 36.8 % of the farmers interviewed had either permitted out or permitted in some cattle from and/or to their kraals in the six months preceding the study (**Table 22**). Out of these farmers, 21.7 % had moved cattle within the same dip tanks, 42.4 % of them were involved in moving cattle within dip tanks in the same sub regions, 31.0 % of them were involved in moving cattle within their regions while the rest, being about 13.1 % of the farmers, moved cattle beyond their regions (**Table 21**). Of the farmers interviewed, those that had moved cattle within their dip tanks, within dip tanks in their sub region, within dip tanks in their region and in dip tanks outside their regions were 7.7 %, 15.6 %, 11.4 % and 2.13 % respectively.

**Table 21** Overall cattle movements

Type of Movement	Overall cattle movement (in and out)				
Origin/Destination	Permitted in	Permitted out	Permitted in and out	Proportional Percentage	Overall cattle movement
Local dip tank	31	30	61	20.9	7.7
Sub regional dip tank	55	67	123	42.2	15.6
Regional dip tank	46	44	90	30.9	11.4
National dip tank	9	8	17	5.8	2.13
<b>Total</b>	<b>141</b>	<b>149</b>	<b>291</b>	<b>100</b>	<b>36.8</b>

### 3.8.4 Consumption patterns

None of the farmers interviewed owned dairy animals within their kraals, while 88.9 % of them consumed unpasteurized milk and milk products obtained from their lactating beef cattle. Furthermore, 92.9 % of the farmers and their families regularly consumed meat and meat products that had not been inspected by the relevant authorities. Of the latter, 100 % consumed all parts of the carcass including high risk organs such as the head and visceral organs, without removing any lymph nodes. Furthermore, 94.8 % admitted to practicing some form of undercooking of the meat, through cooking the meat over an open fire, a popular way of cooking during cultural feasts and event. Lastly, 68.1 % of the farmers interviewed admitted to regularly consuming meat from animals that died on their own whenever it was available (**Table 22**).

**Table 22** Consumption behavior for farmers and their immediate families

Consumption behavior	Number of farmers		Percentage	
	Yes	No	Yes	No
Consumption of unpasteurized milk and milk products	702	87	88.9	11.1
Consumption of uninspected meat and meat products	733	56	92.9	7.1
Consumption of high risk parts i.e. visceral organs and head	733	56	100	0
Failure to remove lymph nodes	733	56	100	0
Undercooking of meat i.e. braaing	695	94	94.8	5.2
Consumption of meat from animal that died on their own whenever available	499	290	68.1	31.9

### 3.8.5 BTB awareness

While 98.4 % of the people interviewed were aware of TB as a serious human diseases, especially to HIV/AIDS patients, only 5.7 % were aware of BTB as a cattle disease and 2.9 % were aware of its zoonotic potential, with 94.3 % of the farmers completely ignorant of BTB as a cattle disease and 97.1 % unaware that it is a zoonosis. None of the farmers had put in place measures to control BTB in their herds or the entry of BTB into their herd. Furthermore, none of the farmers had measures in place to protect themselves from being infected by *M. bovis* from their cattle (**Table 23**).

**Table 23** Farmers responses on their knowledge and awareness of BTB

Factor	Number of Responses		Percentage	
	Yes	No	Yes	No
Awareness of human TB	776	13	98.4	1.6
Awareness of BTB as a cattle disease	45	744	5.7	94.3
Awareness of BTB as a zoonosis	23	766	2.9	97.1
Awareness of BTB as a disease of other domestic animals	14	775	1.8	98.2
Awareness of BTB as a disease of wildlife	9	780	1.1	98.9
Awareness of possibility of transmitting BTB from cattle to other domestic animals and vice versa	11	778	1.4	98.6
Awareness of the potential transmission BTB from wildlife to cattle and vice versa	18	771	2.3	97.7
Awareness of potential to contracting BTB from food of cattle origin	4	785	0.5	99.5
Awareness of contracting BTB from cattle by other means	5	784	0.6	99.4
Presence of wild animals in vicinity	88	781	11.2	88.8
Rearing other domestic animals in vicinity of cattle	424	365	53.7	56.3
Mixing of cattle with domestic animals and wild life	316	758	40.1	59.9
Exposure to educational material on BTB and training	8	781	1.01	98.99

## Chapter 4

# Discussion

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### 4.1 General

The present study is the first to investigate the prevalence of BTB in the cattle population in selected dip tanks in Swaziland. Two methods were used to detect BTB in cattle, being the commonly used Comparative Intra-dermal Skin Test and the IFN- $\gamma$  test, in the form of the Bovigam Test. Both the standard IFN- $\gamma$  (1G) and the new, advanced IFN- $\gamma$  (2G) Tests.

The CIST was used on almost all animals tested while the IFN- $\gamma$  (1G) Test was used in only seven selected dip tanks. While it would have been ideal to test all animals with both tests, financial constraints limited the use of the IFN- $\gamma$  test to seven dip tanks as the test kits and the added laboratory work make it to be much costlier than the CIST. The principal test method in this study was CIST while the IFN- $\gamma$  was used as a secondary complementary test in the seven dip tanks. The new IFN- $\gamma$  (2G) Test was used in selected animals which had been tested for BTB using both the CIST and IFN- $\gamma$  (1G) Test. This test was used as an additional complementary test over the CIST and the IFN- $\gamma$  (1G) tests.

This study is also the first to investigate the risk factors to farmers associated with infection with *M. bovis*. This was achieved through a questionnaire survey whose main objective was to obtain data about cattle populations, movement patterns and disease control measures, cattle management with specific reference to disease management, consumption patterns with regard to animal products such as milk and meat. Abattoir surveillance and a subsequent trace back of the animals identified as BTB positive at *post mortem* inspection was used to identify the dip tanks of study.

### 4.2 Comparative Intra-dermal Skin Test (CIST)

The target for the study was to test at least 10 % of the animals in the study population which is in line with other studies. In this study 10.3 % of animals in the study population were tested, meaning that the target was achieved overall, although in some dip tanks less than 10 % of the animals were tested. This was due to animals not returned for reading on the third day after inoculation of tuberculin. A total of 137 (10.07 %) of the tested animals were not present on the designated day for the reading of the CIST, giving an overall non-compliance rate of 10.16 %.

Elukwatini dip tank had the highest number of cattle owners that did not comply with the requirements for the CIST, with 28 out of 92 (30.44 %) inoculated animals not being returned on the designated day for the reading of the test. In total sixty-three herds (12.5 %) of the 504 herds in the study population herds could not be tested.

While non-compliance has been reported to be a significant shortcoming of CIST in other studies, the success rate in this study was excellent. This challenge had been anticipated and a slightly higher number of cattle than required had been sampled in each dip tank. Tschopp *et al.* (2005) suggested that in case animals do not show up on the day of the reading of the skin test, a house-to-house search, or rather, as in this case, a kraal-to-kraal search, of the implicated animals should be done, the animals found and be read. While this might be feasible in a village set up or in some private farms, it could not have been feasible in this study as the rural set up in the dip tanks involves homesteads with kraals in an 8-10 km radius and the cattle could be anywhere in that area. It would require a lot of time and effort to locate them. Furthermore, Tschopp *et al.* (2005) and co-investigators employed an incentive system to encourage farmers to return their cattle for reading of the test, in their case being the drenching of cattle with anti-parasitic drugs for free. This would have certainly improved the compliance rate in this study to further reduce the number of absent animals during reading of the CIST test but was not implemented due to a lack of resources.

The requirement to handle cattle, other domestic animals and wildlife on two separate occasions, 72 hours apart, and the low sensitivity of the test, are major short comings of the skin test for the diagnosis of BTB (Ramirez-Villaousca *et al.*, 2010). At a cut-off for the bovine bias  $\geq 4$  mm, the sensitivity of the skin test is 59 % while at a cutoff point for the bovine bias  $\geq 2$  mm, the sensitivity of the test is 69 %.

The results of the study indicate that bovine tuberculosis is endemic in the study population, with 10.88 % of the herds tested having at least one BTB positive animal. However, it should be noted that this prevalence of BTB in the population of study, at 6.75 %, is relatively low compared to that reported by Tschopp *et al.* (2008) in Ethiopia, who found that 67 % of herds tested had at least one animal positive for BTB. This would suggest that the BTB endemic in the population of study, while relatively high, is not as serious as it is in other regions in Africa.

Ameni *et al.* (2008) attributes this low transmissibility of BTB in herds to particular management systems, characterized by lack of housing of cattle at night, communal grazing and watering with little food supplementation, lack of overcrowded pastures and low herd sizes. This is

contrasted with management systems characterized by housing and keeping cattle in close contact throughout the year in poor ventilated houses, communal grazing, and watering, in overcrowded pastures and large herd sizes. In the study population, the management system could be said to be more inclined to the former than the latter. In such a system, Tschopp *et al.* (2008) suggest that the main mode of transmission amongst the livestock is cow-to-calf, with calf being infected during suckling and carrying the infection to adulthood.

In this study, the cut-off for a test positive result was set at a bovine bias  $\geq 4$  mm as per the recommendations of the World Organization for Animal Health. The test performance of the CIST can be affected by local conditions due to a number of factors such as the presence/absence and level of environmental Mycobacteria (Awah-Ndukum *et al.*, 2012). Hence it is important that while emphasis is placed on the OIE recommended cut-off point, the cut-off point of a study in a particular geographical region be set according to local conditions (Awah-Ndukum *et al.*, 2009). This is done by using the CIST for BTB diagnosis in combination with the slaughter of affected animals followed by detailed *post mortem* examination for the presence of BTB lesions to determine disease status.

The sensitivity of the CIST at a cut-off of bovine bias  $> 2$  mm is 69 % at a confidence interval of 95 %, while it is 59 % at a bovine bias of  $> 4$  mm. The specificity of CIST at these cut-off points remains identical at 97 % in certain specific conditions (Ameni *et al.*, 2009). Consequently, other studies of BTB prevalence using CIST have used a cut-off of bovine bias  $> 2$  mm to take advantage of the higher sensitivity at the same specificity (Ameni *et al.*, 2009; Tschopp *et al.*, 2008).

Awah-Ndukum *et al.* (2012) used CIST to estimate the prevalence of BTB in cattle in Cameroon at three cut-off points for the test positive animals, being at  $> 4$  mm,  $> 3$  mm and  $> 2$  mm. At a cut-off point of  $> 3$  mm for BTB test positive animals, this study found the number of test positive animals to be 112 while the number of inconclusive animals was 42. The number of animals that tested negative remained the same at 1 177. The number of positive herds was 61 while the number of inconclusive herds was 35. The number of positive male and female animals respectively was 78 and 62, while the number of inconclusive male and females changed to 15 and 27 respectively. The BTB prevalence was 8.4 %.

The increase in the number of BTB test positive animals at a lower cut-off point is in line with the findings of Ameni *et al.* (2008) and Tschopp *et al.* (2010) who all observed an increase in BTB test positive animals at a lower cut-off point for test positive animals. From these observations,

it can be safely concluded that lowering the cut-off point of test positive animals increases the BTB prevalence, as some of the previously inconclusive animals become test positive at the lower cut-off point.

In this study, a lower cut-off point could not be evaluated scientifically by slaughter and culture confirmation of animals with skin test results of 3-4 mm but that the significant number of suspect animals found in this range could be an indication of a higher sensitivity associated with a lower cut-off value. However, the unknown and potentially adverse impact on specificity must not be neglected.

While this study used the recommended OIE cut-off point of  $\geq 4$  mm, the observed changes in the number of BTB positive cattle when the cut-off is slightly lowered to  $\geq 3.0$  mm are statistically significant and cannot be ignored. It is possible that some of the test inconclusive animals are actually test positive. On the other hand, the observed number of cattle that are avian reactors is significantly high, at 48.9 % of all the cattle that were tested. Due to this high percentage of avian reactors, the cut-off in the country should not be lowered without a validation of CIST in Swaziland. This means that all BTB studies using CIST should base the cut-off on the OIE recommended cut-off before the validation of CIST. Currently, lowering the cut-off point could lead to a reduction in the specificity of the skin test.

The avian bias is an outcome of the skin test characterized by a net increase in the skin thickness in the shaved area inoculated with avian PPD as opposed to the shaved area inoculated with bovine PPD. In this study, 652 or 48.9 % heads of cattle coming from 233 or 52.83 % herds showed an avian bias, which was almost half of the total animals tested. This indicates the presence and potential interference of other Mycobacteria, such as environmental Mycobacteria, which could affect the performance of the skin test, leading to false positive results.

Removing the number of cattle with an avian bias from the total number of cattle that are negative for BTB in CIST gives the number of cattle that are true negatives. There were 525 heads of cattle that were true negatives constituting 39.38 % of the animals tested.

In the case of an inconclusive test result, it means that for that particular animal, CIST could not conclusively determine the BTB status. Shirima *et al.* (2000) reported that some animals which were classified as inconclusive during CIST yielded typical BTB lesions when slaughtered and

examined at *post mortem*, and *M. bovis* was isolated when samples from the lesions were cultured. However, in this study, the inconclusive reactors could not be followed up and hence the true prevalence of BTB in the population of study remains unknown.

For purposes of culling all BTB positive animals, the inconclusive animals must be treated as suspect animals and must be retested at least within 60 days of the initial test, as recommended by Shirima *et al.* (2000).

The study found that 88.3 % of the animals tested were BTB test negative. Test negative animals includes true negative animals, those animals that are not infected by *M. bovis* and false negative animals, those animals that are infected by *M. bovis* but the test fails to identify them as infected.

The BTB prevalence of 6.75 % observed in Swaziland was found to be moderate compared to other BTB prevalences found in other countries, as indicated by **Table 1**.

The prevalence calculated above may be deemed to be an apparent prevalence as the test used in the study does not capture all infected animals (Ameni *et al.*, 2008). A test that is capable to capture all infected animals must have specificity and a sensitivity of 100 % and 100 % respectively. Hence the calculated prevalence underestimates the number of animals that are true positive (Ameni *et al.*, 2009). The true prevalence has been calculated in this study. At a cut-off point of  $\geq 4$  mm for test positive animals, it was found to be 12 %, and at a cut-off point of 3.0 mm for test positive animals, it was found to be 12.7 %.

According to **Table 16**, the P-values for the sex of an animal, dip tank type and herd size group are statistically insignificant, as it is greater than 0.05 (i.e.  $0.05 < P$ ) (Xiong *et al.*, 2006) and hence the herd size group, the dip tank type and the gender of an animal are seemingly not statistically significantly associated with its BTB status. The P-values for Hhohho and Shiselweni are statistically insignificant since they are also greater than 0.05, while the P-value for Manzini is statistically significant, as it is lower than 0.05 (i.e.  $0.05 > P$ ) in comparison to the reference, which is the Lubombo region. Being in the Manzini region for an animal maybe associated with it's testing positive for BTB. The odds of an animal in the Manzini region testing positive for BTB are four times that of an animal in the Lubombo region. There is a 300 % chance for an animal in the Manzini region testing positive for BTB compared to the Lubombo region. The odds of an animal in the Shiselweni region testing positive for BTB are two times

that of an animal in the Lubombo region and there is a 100 % chance for an animal in the Shiselweni region testing positive for BTB compared to the Lubombo region. However, these odds are not significant as the P-value for the Shiselweni region is not significant. The odds of an animal in the Hhohho region testing positive for BTB are less than that in the Lubombo region but this is also insignificant as the P-value for the Hhohho region is insignificant.

#### 4.2.1 Culture results

Several studies have indicated that not all animals infected by *M. bovis* have gross lesions that are visible at the tissues that are inspected at slaughter and *M. bovis* has been isolated from lymph nodes and lungs of carcasses that have no visible gross lesions (Asseged *et al.*, 2004). Asseged *et al.* (2004) further estimated that at least 10 % of culture positive animals have no gross lesions for BTB.

In this study, samples were taken from lesions typical of BTB. Eight animals identified as BTB positive through CIST could be recruited for slaughter. Gross lesions typical of BTB were found in the tissues inspected at *post mortem*. *M. bovis* was isolated following culture using standard methods in seven of these eight animals.

The eighth animal is a minor cause of concern as no *M. bovis* was isolated on culture even though it tested positive on CIST, had BTB lesions on *post mortem* inspection and tested positive when stained for acid fast positive organisms. On face value, the negative culture results would look like a contradiction to the other results. However, Asseged *et al.* (2004), state that the lack of viable mycobacteria in calcified lesions of BTB may cause negative culture results in animals that are BTB positive.

Furthermore, Rua-Domenech *et al.* (2010), state that some animals maybe infected with *M. bovis* but have no visible lesions and/or yield negative results on culture. In most cases, these animals have no clinical disease even though they are infected because they are able to temporally contain the bacilli in a condition of latency (Rua-Domenech *et al.*, 2010). This would explain a case of an animal being positive on the CIST but have negative culture results, as in this study.

The study relied on the generosity of the owners of the BTB positive heads of cattle to offer them for slaughter, at no charge to the study, for *post mortem* inspection and the subsequent

collection of samples for culture. Two owners out of 48 owners with BTB positive heads of cattle offered eight heads of cattle out of 90 heads of cattle that were BTB positive i.e. a herd has one legal owner. On these animals, samples were collected from lesions typical of BTB for culture to confirm the presence of *M. bovis* in the study population.

### 4.3 IFN- $\gamma$ 1G (Bovigam) Test

As stated above, one of the limitations of CIST is the low sensitivity of the test (Ramirez-Villaousca *et al.*, 2010). Furthermore, the host's responses to *M. bovis* infection are variable at the different severity stages of pathology of the disease, hence the use of tests which measure cellular and antibody responses may help for the maximum detection of infection (Ameni *et al.*, 2010). Due to this fact, Ameni *et al.*, (2010), suggest the use of more than one test as this leads to the detection of the maximum number of infected animals. To overcome these two challenges, one may consider increasing the rate of testing for BTB using CIST and to use additional test, such as the IFN- $\gamma$  (1G) test (Ramirez-Villaousca *et al.*, 2010) and the lateral flow assay (Ameni *et al.*, 2010). Hence in this study, in seven dip tanks, cattle that had been tested for BTB using CIST were further retested with the commercial IFN- $\gamma$  (1G) Test. A few animals could not complete CIST but had already been bled and hence their results were available only for the IFN- $\gamma$  (1G) test.

When the IFN- $\gamma$  (1G) test is used to diagnose BTB using both avian and bovine PPD, derived from *M. avium* and *M. bovis* respectively, as antigens, the assay can detect infections at an earlier stage than the skin test (Aagaard *et al.*, 2010). However, the IFN- $\gamma$  (1G) test used in the study is a modified version as it includes Fortuitum PPD, which is used to discriminate between specific (induced by *M. bovis*) and non-specific (induced by non-tuberculous Mycobacteria) reactors to eliminate the influence of environmental Mycobacteria on positive test results (Michel *et al.*, 2010).

Out of a total of 452 cattle tested in parallel, 33 animals were positive in the CIST but negative in the IFN- $\gamma$  (1G) Test while 13 animals were positive in the IFN- $\gamma$  (1G) but negative in the CIST. Eight animals were positive in both the CIST and the IFN- $\gamma$  (1G) tests.

Aagaard *et al.* (2010) found a good correlation between a positive skin test and an *ex vivo* test such as the IFN- $\gamma$  (1G), although they used the latter test in addition to other *in vivo* tests, but found that at least 20 % - 25 % of the skin test positive animals should be confirmed by the IFN-

$\gamma$  (1G) test. Results in this study indicated that 41 animals were positive in the skin test of which eight also tested positive in the IFN- $\gamma$  (1G) test, resulting in a 19.51 % agreement for test positive results. This indicates a correlation between the two tests to the level found by Aagaard *et al.* (2010). **Table 18** indicates that nine animals were positive in the IFN- $\gamma$  (1G) but negative in the CIST, an observation also found by Aagaard *et al.* (2010). These researchers suggested that these animals, even though negative in the skin test, were probably infected with *M. bovis*. To account for their negative status in CIST, Aagaard *et al.* (2010), suggest that the negative predictive value of the Purified Protein Derivative (PPD) in herds with a relatively high prevalence is low and hence some infected animal maybe missed during routine testing and surveillance with the skin test. Furthermore, Rua-Domenech *et al.* (2010), states that the IFN- $\gamma$  (1G) detects a substantial proportion of cattle that escape detection by CIST as it identifies animals at an earlier stage of infection than CIST. For negative skin test animals, there is low correlation of CIST with the IFN- $\gamma$  (1G) assay.

Rua-Domenech *et al.* (2010), states that due to the dynamics of *M. bovis* transmission, the microscopic size of the early lesions and the time it takes for an animal to mount a detectable immune response, no single ante or *post mortem* test for BTB can be expected, on it's own, to detect every infected herd and every infected animal in such a herd. In this study, this fact is confirmed by CIST detecting 33 BTB positive heads of cattle which are test negative in the IFN- $\gamma$  (1G) assay. The latter test detected 13 BTB test positive animals which were negative in the former test.

However, even though CIST and the IFN- $\gamma$  (1G) are complimentary in testing for BTB in cattle, they have a disadvantage in that they have a low probability of detecting infected cattle in a state of depressed cell-mediated immune response to tuberculin or anergy (Rua-Domenech *et al.*, 2010). This means that even in this study, the two tests could have missed cattle which are chronically infected with *M. bovis*.

The results indicate that CIST detected 41 BTB positive heads of cattle in contrast to the IFN- $\gamma$  (1G) 21 out of a total of 452 heads of cattle that were tested by both tests. On the surface, CIST seemed to have detected more BTB positive cattle than IFN- $\gamma$  (1G). Rua-Domenech *et al.* (2010), states that a proportion of *M. bovis* infected cattle that are positive reactors to the skin test are not detected by the IFN- $\gamma$  (1G) assay.

For BTB eradication programs and for studies where the BTB status needs to be determined, both the skin test and the IFN- $\gamma$  (1G) should be used and any animal that is positive in either test should be considered BTB positive. The two tests could be used complimentary, even though each test has its own disadvantages. While the skin test is labour intensive and requires the handling of animals on two separate days, the IFN- $\gamma$  (1G) requires a well-equipped laboratory within a reasonable distance. It is problematic if the distance to the laboratory is long, and when a high number of cattle are to be bled as it would require time to do the bleeding and transport the blood samples to the laboratory that is far. Hence a challenge and major disadvantage of the IFN- $\gamma$  (1G) is the need to transport the blood as soon as possible, preferably within eight hours, to the laboratory. The challenge of large numbers of cattle to be bled can be overcome by staggering the bleeding or employing several technicians at a time. The challenge of long distances to the laboratory was not a hurdle in this study as the Central Veterinary Laboratory is centrally located and recently built and well equipped with the latest technology advanced equipment. With the small size of the country, distances to the Central Veterinary Laboratory from any point in the country do not exceed 200 km. These two factors, the relatively short distance and the well-equipped laboratory make the IFN- $\gamma$  (1G) test well suited for use as a diagnostic tool to support BTB control in Swaziland.

The IFN- $\gamma$  (2G) or Bovigam 2G is an improvement of the IFN- $\gamma$  (1) or Bovigam 1G test and it is reported to have a higher specificity at a comparable sensitivity ([www.ars.usda.gov](http://www.ars.usda.gov)). Furthermore, it has more flexibility with regard to incorporation of different reagents for stimulation (PPDs, alternative antigens cocktails) and improved ease-of-use ([www.ars.usda.gov](http://www.ars.usda.gov)). The IFN- $\gamma$  (2G) assay uses improved buffers to block cross-reactivity binding, a one component substrate and less washing steps and thus reduces time and cost and allows full automation for high throughput testing needs ([www.ars.usda.gov](http://www.ars.usda.gov)). It can be used as an improved tool for the detection of BTB infected cattle ([www.ars.usda.gov](http://www.ars.usda.gov)).

In this study, out of 89 heads of cattle tested with the IFN- $\gamma$  (2G) assay, three animals tested positive, two of which had also tested positive in the IFN- $\gamma$  (1G) assay. This indicates a 67 % agreement for test positive results. Seven heads of cattle from the 89 had tested positive for BTB in the IFN- $\gamma$  (1G) assay but only five of these tested positive in the IFN- $\gamma$  (2G). This results could either attest to a higher the high specificity of the IFN- $\gamma$  (2G) assay or alternatively, to a lower sensitivity.

#### 4.4 Questionnaire Survey

The number of calves was relatively low and this was attributed to a number of factors, including the strong reliance on passive breeding where mating is largely left to chance meeting of bulls and cows/heifers in the grazing fields as opposed to a scheduled breeding program. Other factors attributed to the low number of calves were low fertility, poor nutrition, high calf mortality, poor calf management and the fact that the study was carried outside of the calving season. There were many more female cattle in almost all the kraals than there were male ones. Most farmers hold on to their female animals in the hope that they would breed and produce more off-springs, but easily dispose of their male animals whenever it is convenient. Steers, oxen and sometimes bulls are usually sold to raise cash or slaughter for sale as meat or for providing meat in cultural and/or other activities.

Of the farmers interviewed, only sixteen or 2.02 % indicated that their animals were pregnant. This low percentage is due to the fact that pregnancy diagnosis is mainly based on the visible physical clinical signs of pregnancy, such as the progressive extension of the abdomen or the progressive enlargement of the udder. Pregnancy diagnosis by qualified personnel is rarely done. Hence only animals in late gestation were reported.

In all the dip tanks studied, 38.97 % of farmers indicated that they had at least one head of cattle that was six years old and above. Aging of cattle is based on visual appraisal and memory, not on actual birth records and scientific aging. This relatively high percentage of old cattle in the population is due to the fact that off-take of cattle, whereby animals that are no longer productive are culled, is still relatively low, with farmers holding on to cattle for sentimental reasons. Such animals are more likely to be infected by BTB as they have a higher cumulative risk over the years to have been exposed to *M. bovis*. In addition, they are most likely to be a source of infection to younger animals.

Eighty-Eight or 11.1 % of the farmers interviewed reported that their animals were sick or undergoing treatment (**Table 18**), with 4.2 % of them already receiving treatment. In 5.8 % of the cases, the farmers stated that the animals have been sick for prolonged period (**Table 18**). While 2.4 % of the farmers stated that their cattle died on their own, 5.3 % claimed that their cattle died because of their sickness while 7.7 % of the farmers stated that they killed their cattle rather than watch them die of their sickness (**Table 18**). The basis for declaring an animal sick were gross clinical observations such as prolonged anorexia, general weakness and/or recumbence, profuse diarrhea, gross respiratory anomalies or any other pathognomic sign or lesion. In most cases, for an animal to be declared sick, it has to show a particular sign for

extended period of time. Diagnosis is made by the farmer and/or the herd boy who in turn report to the Veterinary Assistant. The Veterinary Assistant at each dip tank is the animal health technician at the interface with the farmers and their livestock. The latter usually prescribes drugs and medicine for the farmer to buy and treat the animal. Only in a few cases is a veterinary surgeon consulted. However, some farmers do consult a veterinarian directly at the earliest suspicion of the onset of a disease.

In most of the dip tanks studied, cattle management was basic and passive. Cattle obtained most, if not all, of their feed from grazing and their water from streams, rivers, ponds and earth dams. Cattle were not provided with extra feed or water. In autumn, after crops have been harvested, cattle are allowed to feed on the crop left overs while standing on the field, with a very limited number of farmers collecting the crop leftovers for feeding to cattle deep into winter. In most cases, grazing pastures are communal and hence pasture rotating is not practical.

Sick animals which are not responding to treatment or which have been sick for prolonged period of time without showing signs of recovery, including moribund animals, are slaughtered for human consumption. This is a salvage procedure as more people are keen to purchase meat of slaughtered animals at a higher price than for that of an animal which died on its own, irrespective of the fact that the animal was moribund. This is the most likely fate of animals with BTB in the various kraals, with their products entering the human food chain. A number of farmers reported during the survey that they had killed off animals that had been sick for prolonged periods and the likelihood that some of these animals had BTB is high especially because of the high percentage of herds which tested positive for BTB.

The limited role of veterinarians in disease diagnosis as observed in this study may lead to a number of diseases such as BTB being misdiagnosed. Exposure of farmers, and consequently their cattle, to veterinary expertise is generally protective of the animals from infectious diseases such as BTB (Munyeme *et al.*, 2008). Indirectly, it also protects the farmers from being infected by *M. bovis* from their own cattle. Farmers that consult veterinarians on a regular basis are more likely to get sound advice about the health status of their animals and are more likely to cull diseased animals as a control measure to implement a control strategy at an early stage, and hence reduce the level of BTB infection (Munyeme *et al.*, 2008). Hence this apparent lack of veterinary consultation favours the spread of BTB in the cattle population and exposes the farmers to the risk of infection of *M. bovis* from their own cattle.

In most of the dip tanks studied, cattle management was basic and passive. Cattle obtained most, if not all, of their feed, from grazing and their water from, streams, rivers, ponds and earth dams. Cattle were not provided with extra feed or water.

The apparent lack of provision of extra feed especially during the winter season when grazing pasture have limited fodder and the low number of farmers providing mineral and vitamin supplements to their cattle makes the cattle population to be vulnerable to BTB infection. With *M. bovis* abundant in their cattle population, the farmers and their immediate families are exposed to the risk of being infected.

The use of the same communal grazing areas by cattle of a particular dip tank encourages the spread of BTB through all the animals in that dip tank. However, cattle that rely on grazing all the time are always out grazing on the pasture all the time, as opposed to those who are fed who always congregate at the feeding troughs. Hence grazing ensures that close contact, which would lead to the transmission of BTB, within cattle, is limited. Lack of provision of water and feed at the kraals further limit close contact amongst the cattle, decreasing chances of spreading infection amongst each other.

Tschopp *et al.* (2009) stated that high parasitic loads may decrease the animal 's resistance and make it more susceptible to BTB. At 33.5 %, a significant number of farmers regularly use remedies for the control of internal parasites, although their use is irregular and haphazard at best. This would roughly equate to a third of the cattle population having a reduced parasitic load at one time or the other of the year.

Cattle housing is not practiced and none of the herds were housed but cattle are kept at kraals at night. In the kraals, the cattle are in such close proximity that they favor the spread of BTB from one animal to the other, but not to such extent as when cattle are housed in poor ventilated structures. While grazing, cattle are on the outside but also when kraaled as kraals are enclosures built in the open, hence cattle are outside all the time. This decreases the risk of transmission of BTB within a herd. Calves are kept in calf pens and are rarely kept in structured stables.

Farmers, herd boys and or any other person do not spend the night in close proximity or in the vicinity of the cattle. Close contact with cattle is limited to driving the cattle to grazing areas or watering points or to the dip tanks, occasional handling of diseased or injured animals, and a

few routine procedures such as milking and the use of animals for drought power. The limited close contact of farmers with their cattle makes farmers less likely to contract *M. bovis* from their cattle due to close proximity, eliminating one of the three major sources of *M. bovis* infection for human beings (Ayele *et al.*, 2004).

During the study none of the farmers interviewed kept dairy animals, although a few farmers claimed some of their neighbors reared dairy animals. However, most, if not all, of the farmers admitted that they milked the beef animals on a regular basis when they are lactating, obtaining anywhere between half-a-litre to three litres of milk per animal per day. The milk obtained is used for human consumption as an important integral part of the diet mainly by family members, with only 7.6 % of farmers claiming they produce enough milk to sell the surplus to their neighbours.

The milk is, naturally, not pasteurized before consumption, as the technique is not only not available to the farmers and impractical for them to implement, but is also not familiar to them, meaning that in the event it comes from BTB infected cows, and contains *M. bovis*, consumers face a direct risk of being infected by *M. bovis*. In almost all cases, farmers stated that all members of a family consumed the milk and its products, although in some cases the milk was reserved for the children in the family, meaning the whole family is exposed to the risk of infection by *M. bovis*, including young children and sick family members, some of them who could be HIV positive, with resulting compromised immunity.

The consumption of raw milk was found to be a potential risk to farmers and their immediate families by Tschopp *et al.* (2008) who observed that at least 68 % of human TB patients in Ethiopia had been regularly consuming raw milk from their cattle.

One of the survey outcome was that 13.7 % of farmers stating that they boiled the milk before consumption as a standard procedure, which is a very encouraging finding that can go a long way in reducing the infection risk of farmers and their immediate families from *M. bovis*. Michel *et al.* (2011) found that routinely applied food processing protocols involving cooking, such as boiling the milk, and drying appear effective in killing *M. bovis* in edible products of naturally infected buffalo and kudu. There is no reason to suggest that these cooking protocols cannot kill *M. bovis* in edible products of naturally infected cattle such as milk. The advantages of pasteurization over boiling is that the appropriate temperature, pressure and duration are achieved over several cycles, meaning that those pathogens which are not destroyed in the initial cycle are destroyed in subsequent cycles. Even, those farmers that boil the milk are still

exposed to some degree of some risk of infection by *M. bovis* because it is doubtful that the boiling is at a high enough temperature, duration and pressure to fully deactivate the *M. bovis* and other pathogens, if present in the milk.

In their study, Michel *et al.* (2011) boiled tissues for a minimum of ten minutes and could not isolate *M. bovis* from naturally infected animals. The minimum duration that milk should be boiled for the effective killing of all *M. bovis* pathogens has not been determined.

Common products derived from milk are 'emasi' (sour milk) and 'umlaza'. These products are obtained by incubating milk at room temperature in specially designed calabashes, allowing the milk to ferment naturally. As the milk ferments to become "emasi" and "umlaza", acid is produced and has a sterilizing effect on pathogens in the milk, including *M. bovis*. Michel *et al.* (2011) found that the survival rate of *M. bovis* in sour milk maintained at 20 °C was significantly higher compared to equivalent samples kept at 33 °C. Unfortunately, the former temperature is around the temperature at which sour milk is traditionally produced, meaning that there is a high likelihood of *M. bovis* being present in the sour milk.

Several studies have indicated that *M. bovis* survive only for a limited time in cultured milk. However, Michel *et al.* (2011), found that in traditionally prepared sour milk, *M. bovis* has been shown to survive, and potentially pose a food safety risk, for at least part of or the entire duration of the product's shelf life. Michel *et al.* (2011) isolated *M. bovis* pathogens in sour milk up to four days, and in some cases up to two weeks, after inoculation, suggesting that sour milk could contain viable *M. bovis* pathogens for that period. Hence the duration of culturing the milk could be a major drawback in rural areas. Due to harsh economic conditions in the rural areas, "emasi" is an important and essential component of the diet. This tends to put a lot of pressure on the culturing process, with many farmers and their families consuming the products after one or two days, when not enough acid has been produced to destroy the *M. bovis* pathogens, exposing the consumers to the risk of infection. Farmers need to consume the milk after at least four days of culturing, where it is estimated that most, if not all, the *M. bovis* pathogens have been destroyed.

Of the farmers interviewed, 92.6 % stated that from time to time, they slaughter cattle from their herds for human consumption. The frequency of this is very variable depending on the purpose for the slaughter. In most cases, these are social activities such as weddings, parties, traditional activities especially the payment of dowry and family gatherings. No *ante-mortem* inspection is conducted on the live animal and no *post mortem* inspection is performed on the

carcass. Lymph nodes are never removed from the meat before the meat is consumed. In some cases, all members of the community are given free access to the meat, especially during social and traditional events. All parts of the carcass are consumed, including high risk organs such as the head and the visceral organs. The practice of direct slaughter of cattle without *ante* and *post mortem* inspection, with the consumption of high risk visceral organs and the head without the removal of lymph nodes exposes consumers to the risk of infection with *M. bovis*. This is confounded by the fact that some cattle in the dip tanks of study are already infected.

An extenuating factor in the risk of being exposed to infection by *M. bovis* is the undercooking of meat obtained from cattle slaughtered without *ante-mortem* inspection. All farmers interviewed admitted that they practice “braaing”, the direct cooking of large chunks of meat over an open fire, over a relatively short period of time, the average being about five minutes. In certain cultural practices, like payment of dowry, it is customary to prepare very large portions of meat in this way. While the meat is very tasty, it is virtually undercooked, especially at the core, and hence if *M. bovis* pathogens are present, they would be left intact, exposing the consumer to infection.

This is in line with findings by Ayele *et al.* (2004) who pointed out that the consumption of poorly heated meat as one of three main sources of infection for human beings, together with the consumption of unpasteurized milk and close contact.

Furthermore, 68.1 % of the farmers stated that they consume meat from animals found dead, without a diagnosis of the cause of death. This can be attributed to poverty, amongst other factors, but ultimately exposes the consumer to infection by *M. bovis* if the animal is infected.

The consumption of raw, unpasteurized/unboiled milk, consumption of uninspected meat including high risk organs and meat from dead animals as well as the undercooking of meat is the predominant consumption habits exhibited by the farmers and their families in the study, which are potential risk factors that expose them to infection with *M. bovis*. Tschopp *et al.* (2008) found similar consumption habits, although undercooking of meat was replaced by consumption of raw meat. However, Tschopp *et al.* (2008), while agreeing that the consumption habits were potential risk factors, found that they were not statistically significant, when correlated with actual human TB cases caused by *M. bovis*. On the contrary, Ameni *et al.* (2003), found that farmers’ consumption habits were statistically significant. The former researcher suggests that further studies on the risk factors on BTB infection requires case control studies with

confirmed *M. bovis* infections and a thorough knowledge of all possible risk factors for humans is a prerequisite for national BTB control programs (Tschopp *et al.*, 2008).

There was a significant level of cattle movement noted as 18.8 % of interviewed farmers reported that they had received cattle into their kraals while 18.0 % had permitted out cattle from their kraals. As illustrated in **Table 16**, cattle movements were either from or to a dip tank in the same sub region or other sub-regions and regions of the country. At 7.1 % for cattle permitted in and 8.5 % for cattle permitted out, a majority of cattle movements was to or from dip tanks within the same sub region as the dip tank of origin or destination respectively. A number of reasons were put forward for the in and out movement of cattle from kraal to kraal, chief amongst them being the payment of dowry and customary fines, especially the penalty for making an unmarried girl pregnant, as well as sales to cattle agents and other parties who purchase cattle for various reasons including slaughter in butcheries for financial gains. The Animal Diseases Act of 1968, Swaziland, stipulates that no animal must be moved from its dip tank if it is sick or diseased but interestingly does not enforce the clinical examination of cattle before being moved, and hence all these cattle that are moved are not screened for the presence of diseases such as BTB. This facilitates the spread of BTB amongst the dip tank as the introduction of a diseased animal in a population is a major form of spread of BTB.

The introduction of cattle into a herd can introduce cattle infected with BTB even in an area that is already endemic with BTB (Ramirez-Villaousca *et al.*, 2010). Introducing BTB infected cattle in a herd can cause persistence of infection as the cattle are re-exposed and probably re-infected with *M. bovis* each time a new animal is brought into the herd (Ramirez-Villaousca *et al.*, 2010). Hence the increased cattle movements facilitate the introduction of cattle infected with BTB into herds and pose a risk of causing herds persistently infected with BTB. Ramirez-Villaousca *et al.* (2010) also found out that introduction of cattle into herds, through purchases from markets, other farms, etc., is a risk for BTB introduction and persistence especially in the absence pre-movement testing as a statutory requirement (Ramiez-Villaousca *et al.*, 2010). Although pre-movement testing as a statutory requirement for all cattle to be moved is recommended as a means to curb the introduction of BTB infected cattle into herds, this might not be a viable solution due to lack of resources.

Wedlock *et al.* (2002) stated that while the test and slaughter method is effective in the control of BTB, movement control from infected farms should also be equally controlled in order to control BTB infections. In this case, that would equate to the control of movement from infected dip tanks or herds to uninfected dip tanks or herds. This will be challenging as the BTB status

of most dip tanks is not known. Phillips *et al.* (2002) also attributed cattle movement as the major mode of transmission of *M. bovis* especially in areas which are devoid of wildlife populations (Phillips *et al.*, 2002).

While many of the farmers claimed to be conversant with the common cattle diseases, a majority of the farmers who were interviewed alluded to the fact that there were not aware of BTB as a significant cattle diseases and an important zoonosis.

Farmers' knowledge of BTB as important cattle diseases and as a zoonosis was generally poor. This finding has negative implications as the lack of knowledge would indirectly leads to farmers being exposed to infection. A very serious and widespread awareness campaign should be embarked on to improve farmers' knowledge and awareness of BTB.

Ameni *et al.* (2003) when assessing BTB public health implications using a questionnaire survey in Central Ethiopia, found that 35 % of respondents knew about BTB while only 32 % of the respondents were aware that it could be transmitted from cattle to humans. Still, the researchers concluded that farmer's awareness about BTB and its transmission was generally poor (Ameni *et al.*, 2003). The researchers found a more positive awareness than this study but their conclusion about poor awareness is similar to those of this study. It would seem poor awareness about BTB amongst farmers is a generalized problem not only in Swaziland but also elsewhere on the African continent.

## Chapter 5

# Conclusion

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BTB is present in the cattle herds in Swaziland and the estimated prevalence of BTB in the study population, as determined by the tuberculin skin test, in this cross-sectional study was found to be 6.75 %. The true prevalence of BTB in the study population is probably higher, based on the diagnostic test performance of the CIST, which is estimated at 70 % sensitivity and up to 97 % specificity, meaning that the test is likely to miss many truly infected animals (false negatives).

At 6.75 % the prevalence of BTB in the study population is significantly high to consider BTB an important disease of cattle, posing an infection risk on other species and humans in Swaziland.

During structured interviews, farmers were found to lack basic knowledge of BTB as an important cattle disease. The high risk consumption behavior with regard to consumption of high risk milk and meat products, coupled with the low awareness of BTB and the lack of mitigating control measures are reasons to consider *M. bovis* as a serious threat to public health in Swaziland.

The study revealed minimum veterinary consultation by farmers and a limited provision of veterinary services such as meat inspection and clinical services. It is evident that the veterinary health technicians are at the interface with the farmers and their livestock. Their role in the control of diseases such as BTB and others is crucial and hence needs to be empowered as much as possible to ensure that they are well equipped for these challenges.

There is a need for the Veterinary Department of Swaziland to review and implement the existing BTB Control Program and, most importantly, there is a need to investigate *M. bovis* infection in humans.

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## Annex 1 :

### Questionnaire that was used in the Questionnaire Survey

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**Title: The prevalence of bovine tuberculosis and associated risk factors for humans in Swaziland**

**Name of Diptank** \_\_\_\_\_ *(Please Tick correct answer where applicable)*

#### **Part A: General Information**

- |    |  |                  |           |              |
|----|--|------------------|-----------|--------------|
| 1. | Number of Cattle in Kraal                            |                  |           |              |
|    | None----   | Less than 10---- | 10-30---- | above 30---- |
| 2. | Number of adult cattle (animals above 30 months old) |                  |           |              |
|    | None----   | Less than 10---- | 10-30---- | above 30---- |
| 3. | Number of calves/weaners (animals below 30 months)   |                  |           |              |
|    | None----   | Less than 10---- | 10-30---- | above 30---- |
| 4. | Number of male animals                               |                  |           |              |
|    | None----   | Less than 10---- | 10-30---- | above 30---- |
| 5. | Number of female animals                             |                  |           |              |
|    | None----   | Less than 10---- | 10-30---- | above 30---- |
| 6. | Number of Pregnant animals                           |                  |           |              |
|    | None----   | Less than 10---- | 10-30---- | above 30---- |
| 7. | Number of Lactating animals                          |                  |           |              |
|    | None----   | Less than 10---- | 10-30---- | above 30---- |
| 8. | Number of animals over 6 years old                   |                  |           |              |
|    | None----   | Less than 10---- | 10-30---- | above 30---- |

#### **Part B: General health Status of cattle**

***All questions refer to the last 6 months unless otherwise stated***

- |    |                                       |                 |         |             |
|----|---------------------------------------|-----------------|---------|-------------|
| 9. | Number of currently sick/ill animals. |                 |         |             |
|    | None----                              | Less than 3---- | 3-5---- | above 5---- |

10. Number of animals currently receiving treatment/medication.  
None---- Less than 3---- 3-5---- above 5----
11. Number of animals that recovered from sickness/illness.  
None---- Less than 3---- 3-5---- above 5----
12. Number of animals that died of sickness/illness.  
None---- Less than 3---- 3-5---- above 5----
13. Number of animals that show prolonged sickness and recovered.  
None---- less than 3---- 3-5---- above 5----
14. Number of animals that showed prolonged sickness and did not recover.  
None---- less than 3---- 3-5---- above 5----
15. Number of animals that showed general body loss and emaciation.  
None---- Less than 3---- 3-5---- above 5----
16. Number of animals that always lag behind others.  
None---- less than 3---- 3-5---- above 5----
17. Number of cattle that died on its own.  
None---- Less than 3---- 3-5---- above 5----
18. Number of cattle that were killed following sickness.  
None---- Less than 3---- 3-5---- above 5----

**Part C: General cattle management**

19. Are animals provided with extra feed?  
Never---- Sometimes---- always----
20. Are animals provided with water in troughs at kraals?  
Never---- sometimes---- always----
21. Are animals provided with salt licks?  
Never---- sometimes---- always----
22. Are animals dewormed?  
Never---- sometimes---- always----
23. Are animals given extra medication for external parasites?  
Never---- sometimes---- always----

24. Is a veterinary surgeon consulted when animal is sick?  
Never---- sometimes---- always----
25. Is paraveterinarian consulted when animal is sick?  
Never---- sometimes---- always----
26. Are veterinary drugs purchased with a Surgeon's prescription?  
Never---- sometimes---- always----
27. Are calves housed/kept in doors?  
Never---- Sometimes---- always----
28. Are adult cattle housed/kept indoors?  
Never---- Sometimes---- always----

**Part D: Animal products**

29. Number of Dairy cattle in Kraal.  
None---- less than 3---- 3-5---- above 5----
30. Number of neighbouring kraals with Dairy cattle.  
None---- less than 3---- 3-5---- above 5----
31. Are beef cattle being milked?  
Never---- Sometimes---- always----
32. Is milk from beef cattle consumed within the owner's household?  
Never---- Sometimes---- always----
33. Type of milk products produces  
Fresh milk---- Emasi---- Umlaza---- Other----
34. How many days does it take to make Emasi  
Less than 1 day---- 1-2 days---- More than 3 days----
35. How many days does it take to make Umlaza  
Less than 1 day---- 1-2 days---- More than 3 days----
- Is the milk sold to neighbours?  
Never---- Sometimes---- Always----
36. Daily quantities consume  
Less than 1l---- 1-2l---- more than 2l----

37. People who consume milk products  
 Everyone---- Children---- Adults---- Elderly----
38. Is the milk from beef cattle boiled before being consumed?  
 Never---- Sometimes---- Always----
39. Is the milk pasteurized before being consumed?  
 Never---- Sometimes---- Always----
40. Are cattle slaughtered for family consumption  
 Never---- Sometimes---- always----
- If yes, how many animals per year \_\_\_\_\_
41. Are cattle slaughtered for community/traditional purposes/consumption?  
 Never---- Sometimes---- always----
42. Is *ante mortem* performed on slaughter cattle?  
 Never---- Sometimes---- always----
- If yes, by whom \_\_\_\_\_
43. Is *post mortem* examination performed on carcasses?  
 Never---- Sometimes---- always----
44. Is the head consumed?  
 Never---- Sometimes---- Always----
45. Are the visceral organs consumed?  
 Never---- Sometimes---- Always----
46. Are lymph nodes removed from red meat?  
 Never---- Sometimes---- Always----
47. Is the meat braai-ed (cooked on coals)?  
 Never---- Sometimes---- Always----
- If yes, how big are the chunks of meat.....
48. If meat is braai-ed, for how long ?  
 Less than 10 mins---- 10-20 mins---- More than 20 mins----
49. What happens to animals that die on their own (are they consumed)?  
 Never---- Sometimes---- Always----

**Part E: Cattle movement**

***All questions refer to the last 6 months unless otherwise stated***

50. Are there any new animals in kraal?

Yes---- NO----

51. Are these from the same dip tank?

Yes---- No----

52. Are there from the same sub region?

Yes---- No----

53. Are there from the same region?

Yes---- No----

54. Were the animals purchased for (Please tick)

Lobola---- Breeding---- Traditional activity---- Re-sale----

55. Are there any animals that left the kraal?

Yes---- No----

56. Reason for animals leaving (Please tick)

Lobola---- traditional activity---- sale for slaughter---- Breeding----

57. Did they go to the same dip tank?

Yes---- No----

58. Did they go to the same sub region?

Yes---- No----

59. Did they go to the same region?

Yes---- No----

60. Were animal sold to local dip tanks?

Yes---- No----

61. Were animals sold to SMI?

Yes---- No----

62. Were animals sold to cattle agents?

Yes---- No----

63. Payment method for animals that left kraal.

Cash---- Kind----

**Part F: Bovine Tuberculosis (BTB)**

64. Are you aware of tuberculosis?

Yes---- No----

65. Does it affect cattle?

Yes---- No---- Do not know----

66. Does it affect human beings?

Yes---- No---- Do not know----

67. Does it affect other animals?

Yes---- No---- Do not know----

68. Can it be transmitted from human to animals?

Yes---- No---- Do not know----

69. Can it be transmitted from animals to humans?

Yes---- No---- Do not know----

70. Has the veterinarians/paraveterinarians ever sensitized you about BTB?

Yes---- No----

71. Does prolonged close proximity to cattle transmit disease?

Yes---- No---- do not know----

72. Does consuming affected milk transmit disease?

Yes---- No---- Do not know----

73. Does consuming affected head and visceral organs transmit BTB?

Yes---- No---- Do not know----

74. Does consuming meat transmit BTB?

Yes---- No---- Do not know----

75. Is there anything that is being done to protect yourself against BTB?

Yes---- No---- Not aware----

76. Has any of your cattle suspected/diagnosed as having BTB (tick appropriate)

Yes---- No---- Not aware----

77. Has any of your neighbours' cattle suspected/diagnosed as having BTB

Yes---- No---- Not aware----

78. Would you be concerned if your cattle had BTB

Yes---- No----

**Part G: Miscellaneous**

79. Do you have any of these animals in your kraal?

Sheep---- Goats---- Pigs---- Donkeys----

80. Do the above animals mix with your cattle?

Never---- Sometimes---- always----

81. Do you have any of these wild life in the neighborhood?

Impala---- Impunzi---- wild pigs---- hare----  
Kudu---- Jackal---- mongoose---- skunk----

82. Do they or any other wildlife mix with the cattle?

Never---- Sometimes---- Always----

## Annex 2 :

### An example of the Data Base for the CIST

district	diptank	krall ID	Animal ID	nimal Nam	colour	sex	age	test date
Manzini	Crydon	57		ngabisa	red	F	adult	24.05.2011
Manzini	Crydon	109		mabadlana	red	F	adult	24.05.2013
Manzini	Crydon	23		miss	red	F	adult	24.05.2014
Manzini	Crydon	15		Ngwedle	red	F	adult	24.05.2015
Manzini	Crydon	15		dymane	red	M	adult	24.05.2016
Manzini	Crydon	15		mthokazi	red	F	adult	24.05.2017
Manzini	Crydon	30		Sibuko	red	F	adult	24.05.2019
Manzini	Crydon	92		mcebo	red	F	adult	24.05.2020
Manzini	Crydon	4		Austin	nsundu/wh	M	adult	24.05.2021
Manzini	Crydon	1		messis	nsundu	F	adult	24.05.2022
Manzini	Crydon	31		shodi	bla/whi	M	adult	24.05.2023
Manzini	Crydon	34		mnyamane	Black	F	adult	24.05.2024
Manzini	Crydon	14		ntabamhlo	roan	M	adult	24.05.2025
Manzini	Crydon	27		mhlekazi	yellow	F	adult	24.05.2026
Manzini	Crydon	104		sidv/574	black	F	adult	24.05.2027
Manzini	Crydon	49		lefosi	red/whi	M	adult	24.05.2028
Manzini	Crydon	93		ngabisa	whi/blac	F	adult	24.05.2029
Manzini	Crydon	65		mahlatsi	red/whi	M	adult	24.05.2030
Manzini	Crydon	75		mshini	white	M	adult	24.05.2031
Manzini	Crydon	<b>46</b>		<b>mzambia</b>	<b>nsundu</b>	<b>M</b>	adult	24.05.2032