PREVALENCE, ETIOLOGY, PUBLIC HEALTH IMPORTANCE AND ECONOMIC IMPACT OF MYCOBACTERIOSIS IN SLAUGHTER CATTLE IN LAIKIPIA COUNTY, KENYA.

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

This work is dedicated to:

My dear wife Dorothy, our children Kimathi, Munene and Karimi

and

My parents

The late Mr. M'Akwalu and my loving mother, Sarah Akwalu.

ACKNOWLEDGEMENT

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LIST OF ABBREVIATIONS AND ACRONYMS

AFB	Acid Fast Bacilli
AS	Additional Mycobacteria Species
ASAL	Arid and Semi-Arid Lands
BSC	Bio- Safety Cabinet
CBPP	Contagious Bovine Pleuro-Pneumonia
CC	Conjugate Control
CIDTT	Comparative Intra Dermal Tuberculin Test
CM	Common Mycobacteria
CNS	Central Nervous System
CON	Conjugate
CTRL	Central Tuberculosis Reference Laboratory
CVL	Central Veterinary Laboratories
DEN	Denaturation Solution
DNA	Deoxyribonucleic Acid
DR	Direct Repeat
DVS	Director of Veterinary Services
ECDPC	European Centre for Disease Control and Prevention
EM	Environmental Mycobacteria
EFSA	European Food Safety Authority
FMD	Foot and Mouth Disease
GC	Genus Control
H/C	Heads of cattle
HIV/AIDS	Human Immuno-deficiency Virus/Acquired Immuno-deficiency Syndrome
HYB	Hybridization Buffer
IC	Internal Control

IFNγ	Gamma Interferon Assay
INH	Isonicotinic Acid Hydrazide
KNBS	Kenya National Bureau of Statistics
L-J	Lowenstein-Jensen Media
LNs	Lymph nodes
MAC	Mycobacterium Avium Complex
MDR-CUL	Medical Device Regulation - Culture
MGIT	Mycobacterial Growth Indicator Tube
MOTT	Mycobacterium Other Than Tuberculosis
MTBC	Mycobacterium Tuberculosis Complex
NaOH	Sodium Hydroxide
n.d.	No date
NTRL	National Tuberculosis Reference Laboratory
NTM	Non- Tuberculous Mycobacteria
OADC	Oleic acid, Albumin, Dextrose, Catalase
OIE	Office Internationale des Epizooties
PANTA	Polymixin B, Amphotericin B, Nalidixic acid, Trimethoprim
PBS	Physiological Buffer Solution
PCR	Polymerase Chain Reaction
PM	Post Mortem
PNB	Para- Nitro benzoic acid
PPD-B	Bovine Purified Protein Derivative
PPD-A	Avian Purified Protein Derivative
PPR	Peste des Petits Ruminants
PZA	Pyrazinamide
RFLP	Restriction Fragment Length Polymorphism
RIN	Rinsing Solution

RLU	Relative Lights Units
RNA	Riboxy Nucleic Acid
SITT	Single Intra Dermal Tuberculin Test
STR	Strigent Solution
TB	Tuberculosis
TCH	Thiopene-2-Caroxylic Acid Hydrazide
TST	Tuberculin Skin Test
UC	Universal Control
UV	Ultra – Violet
USA	United States of America
WHO	World Health Organisation
Xg	Times Gravity (Units)
ZN	Ziehl-Neelsen

ABSTRACT

Mycobacteriosis is a chronic or acute systemic granulomatous disease that affects mammals, fish and birds. There are over 150 species recognized in *Mycobacterium* genus. The genus is divided into three groups according to clinical importance: obligatory human and animal pathogens, potentially pathogenic microorganisms (opportunistic) for animals and humans, and saprophytes or ubiquitous microorganisms. In cattle, mycobacteriosis is mainly caused by *Mycobacterium bovis* subsp.*bovis*. Recently Mycobacteria Other Than Tuberculosis (MOTTs) have been isolated from tubeculous lesions in cattle. This study was carried out to determine the prevalence, etiology, public health importance, and economic impact of bovine mycobacteriosis in Laikipia County, Kenya. A cross-sectional study was conducted at Laikipia Municipality Abattoir in Laikipia County between January and July, 2015. Post mortem meat inspection was carried out according to procedures established by legislation (Meat Control Act, cap.356), where samples suggestive of tuberculous lesions from inspected cattle carcasses were taken, trimmed and packed in sterile labelled Falcon tubes, transported to the laboratory and stored at -20 ^{O}C before processing. During processing in a level II laboratory, samples were homogenized and decontaminated. Smears were prepared and stained by the Ziehl-Neelsen method. The samples were then cultured in three different Loweisten-Jensen (L-J) media containing either pyruvate, glycerol or para-nitro benzoic acid. Cultures were incubated for 4-12 weeks. The isolates were then speciated by molecular analysis using PCR. GenoType Mycobacterium MTBC, CM and AS kits (Hains Lifescience, Germany) were used in this analysis. Economic impact of mycobacteriosis at the abattoir was determined by estimating the value of organs and tissues condemned due to presence of lesions. At national level, a desktop study was conducted to collect and collate the information on losses of export opportunities due to bovine tuberculosis threat in Kenya. Trade inquiries for the last ten (10) years were analysed and given monetary values.

One thousand (1000) cattle carcasses were sampled for the study and the prevalence was found to be 21.8% (218/1000) on the basis of gross lesion. Direct ZN staining detected Acid Fast Bacilli (AFB) in 28.9% (63/218) of all the lesions. L-J media isolated 22.9% (50/218) isolates, which were further analyzed for mycobacteria species using PCR. Mycobacteria were identified in 70% (35/50) of the isolates. The isolated mycobacteria were *Mycobacterium fortuitum* at 24% (12/50), *Mycobacterium bovis* subsp. *bovis* 6.0% (3/50), *Mycobacterium shimoidei* 4.0% (2/50) and *Mycobacterium asciaticum*, *Mycobacterium interjectuctum*, *Mycobacterium szulgai*, *Mycobacterium celatum*, *Mycobacterium kansaasii* at 2.0% (1/50) each. *Mycobacterium species* could not be speciated in 26% (13/50) of the isolates and 30% (15/50) isolates were negative for mycobacteria. The overall prevalence of mycobacteriosis in this study was 3.5% (35/1000) [95% CI: 2.53-4.83]. Condemned organs and tissues weighed 422 kilograms which translated to an estimated loss of 168,800 Kenya shillings for the study period. Annual estimated loss was 506,400 Kenya shillings (USD 5,604). At the national level two enquiries failed for the 10 year period under consideration (2008 – 2014) with an estimated economic loss of Kenya shillings 474mln (4.74million USD). This study demonstrated the presence of *Mycobacterium bovis* subsp.*bovis*, *Mycobacterium fortuitum*, *Mycobacterium shimoidei*, *Mycobacterium asciaticum*, *Mycobacterium interjectuctum*, *Mycobacterium szulgai*, *Mycobacterium celatum*, *Mycobacterium kansaasii*, and *Mycobacterium species* as causative agents of mycobacteriosis in cattle in the study area. Further, MOTTS isolated from cattle in this study have been found by other researchers to cause pulmonary tuberculosis in humans. In conclusion, diagnosis of bovine tuberculosis should take into account the pathogenic potential of Mycobacteria Other Than Tuberculosis (MOTTs). Condemnation of organs/tissues and restriction on animal trade due to bovine mycobacteriosis impacts negatively on the national economy.

INTRODUCTION

1.1 Background

Mycobacteriosis is a chronic or acute systemic granulomatous disease that affect mammals, fish and birds. It is a chronic infectious disease, which presents caseous necrotic lesions in tissues and organs of animals (Ishikawa *et al*., 2011). Mycobacteriosis is the only known disease that forms caseous necrosis in animals and humans (chuohoki, 2011). Mycobacteriosis in cattle is caused mainly by *Mycobacterium bovis* subsp.*bovis* but *Mycobacterium bovis* subsp.*caprae*, *Mycobacterium tuberculosis* and *Mycobacterium africanum*, which belong to the *Mycobacterium tuberculosis complex* (MTBC) group have also been reported to infect cattle (Aranaz *et al*., 2004; Bezos *et al*., 2012; Duarte *et al*., 2008; Shitaye *et al*., 2006; Smith, 2003). The main route of infection in cattle is through aerosol exposure, facilitated by close contact between animals (Neill *et al*., 2005). Infectious animals shed *Mycobacterium* through milk, saliva, feces/urine and discharging lesions (Phillips *et al*., 2003). *Mycobacterium* could be transmitted to humans from cattle through the inhalation of cough sprayed from infected cattle, or from handling or consumption of milk contaminated with the mycobacteria (Wedlock *et al*., 2002). Therefore, it has an important public health concern Worldwide especially in developing countries due to unsanitary cultural practices and low hygiene standards (Cosivi *et al*., 1998).

The disease, commonly referred to as bovine tuberculosis or zoonotic tuberculosis, has a Worldwide distribution but is endemic in developing countries where preventive measures are inadequate or lacking. Recently various studies have shown the role of a wide range of Mycobacteria Other Than Tuberculosis (MOTTs) in etiology of mycobacteriosis in cattle. MOTTs are widespread in the environment and aquatic reservoirs. MOTTs have been found to infect animals and humans, causing lesions and symptoms similar to those caused by

obligatory pathogenic mycobacteria, implying their potential role as pathogens (Mirsaeidi *et al*., 2014). However, reports of animal diseases caused by MOTTs are few.

Studies on tuberculosis in animals have concentrated mainly on zoonotic bovine tuberculosis, neglecting the potential pathogenic and zoonotic role of other types of mycobacteria. These non-pathogenic mycobacteria can also induce cross-reactive immune responses that may interfere with tuberculosis diagnostic screening tests, such as the tuberculin test, leading to false positive reactions (Thacker *et al*., 2013). This may hinder diagnosis of zoonotic tuberculosis, affect control/eradication efforts and restrict animal trade.

According to the WHO, (2004) mortality and morbidity statistics included 14.6 million chronic active cases, 8.9 million new cases, and 1.6 million deaths, mostly in developing countries with an expected 1% increase annually. In the developed World, zoonotic tuberculosis is responsible for 5-10% of human TB cases but this varies between countries (Haddad *et al*., 2004). Economic losses due to cattle mycobacteriosis worldwide is estimated at $3billion annually (Thoen *et al*., 2006).

In Kenya, a survey was carried out in two slaughterhouses supplying beef to Nairobi city and its environs which established presence of the mycobacteriosis (Gathogo *et al*., 2012a) with a prevalence of 2% but MOTTs isolated in this study were not characterised. Another study by Kang'ethe *et al*., (2007) reported a 10% prevalence of mycobacteriosis in Dagoretti Division using Single Intradermal Tuberculin Test (SITT).

This investigation was to establish prevalence of mycobacteriosis in cattle, etiology, public health importance and also estimate the economic impact of mycobacteriosis in Laikipia County. Information generated in this study will inform policy makers and possibly help in policies formulation that will guide in controlling mycobacteriosis in cattle.

1.2 Justification

Zoonotic tuberculosis poses serious public health threat as well as economic burden due to losses through reduced productivity of infected animals, animal market restrictions and control and eradication programs. In Africa zoonotic tuberculosis has widespread occurrence. Regionally, the disease has been reported in Somalia, Ethiopia, Tanzania, Burundi and Uganda. In Kenya there has been limited investigation into the occurrence of the disease. MOTTS have been found to infect animals and humans, causing lesions and symptoms similar to those caused by obligatory pathogenic mycobacteria, implying their potential role as pathogens. However, reports of animal diseases caused by MOTTs are few. Studies on tuberculosis in animals have concentrated only on zoonotic bovine tuberculosis, neglecting the potential pathogenic and zoonotic role of other types of mycobacteria. These non-pathogenic mycobacteria can also induce cross-reactive immune responses that may interfere with tuberculosis diagnostic screening tests, such as the tuberculin test, leading to false positive reactions. This may hinder diagnosis of zoonotic tuberculosis, affect control/eradication efforts and restrict animal trade. The information generated in this study will strengthen surveillance and control of bovine tuberculosis through improved diagnostic strategies.

1.3 Overall Objective

This study was carried out to determine the prevalence, etiology, public health importance and economic impact of bovine mycobacteriosis in Laikipia County, Kenya.

1.3.1 Specific objectives

1. Determine the prevalence of mycobacteriosis in slaughter cattle.

2. To isolate various species of mycobacteria in slaughter cattle and to determine their aetiology.

3. To relate and show the zoonotic importance of isolated mycobacteria species.

4. To estimate economic losses due to condemnations of slaughter cattle and estimation of trade inquiries failure at the national level due to mycobacteriosis.

2.0 LITERATURE REVIEW

2.1 Etiology

Mycobacteriosis is a chronic or acute systemic granulomatous disease that affects mammals, fish and bird(OIE, 2009). It is caused by members of the genus Mycobacterium. Currently, the genus *Mycobacterium* comprises of 163 species and 13 subspecies as described in the list of bacterial species with approved names (Khosravi *et al*., 2017).

2.1.1 Classification of mycobacteria

Classification of *Mycobacterium* genus is based on several different factors including growth rate, pigmentation, fatty acid content and pathogenicity. Previously grouping based on pathogenicity, placed *Mycobacterium* species in two groups: tuberculosis and non-tuberculosis mycobacteria (Eisenstadt and Hall, 1995). Non-tuberculosis mycobacteria (NTM), which presently been changed to Mycobacteria Other than Tuberculosis (MOTTs) are further characterized into slow and rapidly growing mycobacteria, with rapid-growers being those that produce grossly visible colonies from dilute inoculum in solid media, in less than 7 days under optimal nutrient and temperature regimes. Slow-growers are those taking over 7 days to give visible colonies (Lévy-Frébault and Portaels, 1992). More recent: obligatory human and animal pathogens, potentially pathogenic microorganisms (opportunistic) for animals and humans, and saprophytes or ubiquitous microorganisms (Stanford and Stanford, 2012).

Obligatory pathogens include species belonging to the *Mycobacterium tuberculosis* complex (MTBC) that include *Mycobacterium bovis* subsp.*bovis, Mycobacterium bovis* subsp.*caprae, Mycobacterium tuberculosis, Mycobacterium africanum, Mycobacterium bovis BCG, Mycobacterium canetti, Mycobacterium microtti, Mycobacterium pinnipendi* and *Mycobacterium liprae* (Alexander *et al*., 2010). *Mycobacterium tuberculosis, Mycobacterium*

africanum, Mycobacterium caneti are predominantly human pathogens (Bezos *et al*., 2012; Meyer *et al*., 2008), although cattle infections have been reported (Romero *et al*., 2011; Shitaye *et al*., 2006). *Mycobacterium liprae* causes leprosy in humans (Collins, 2011; Haddad *et al*., 2004). *Mycobacterium pennipedii* and *Mycobacterium microti* affects marine mammals (Kiers *et al*., 2008), rodents (Cavanagh *et al*., 2002) and branded mongoose[Mungos mungo] (Alexander *et al*., 2010). The MTBC group is considered a subspecies of genus *Mycobacterium* and family *Mycobacteraceae* with a 99.9% similarity at nucleotide level, but differ widely in terms of their host tropisms, phenotypes and pathogenicity (Brosch *et al*., 2002; Smith, 2003). All MTBC bacteria evolved from a common ancestor and have identical 16S rRNA sequences (Brosch *et al*., 2002; Smith, 2003; Thoen *et al*., 2006).

The potentially pathogenic mycobacteria are represented by the *Mycobacterium avium* complex (MAC) consisting of closely related species and subspecies including *Mycobacterium avium* subsp. *avium, Mycobacterium avium* subsp. *paratuberculosis, Mycobacterium avium* subsp. *hominissuis, Mycobacterium intracellulare, Mycobacterium sylvaticum, Mycobacterium colombiense, Mycobacterium bouchedurhonense, Mycobacterium timonense, Mycobacterium chimaera, Mycobacterium arosiense, Mycobacterium yongonense*, and *Mycobacterium marseillense* (Cayrou *et al*., 2010). The potentially pathogenic species are found in susceptible hosts and cause disease on special circumstances.

M. avium subsp. *avium, M. avium* subsp. *hominissuis* and *M. intracellulare* are responsible for infections especially in birds and pigs (Cayrou *et al*., 2010). *Mycobacterium avium* subsp. *hominissuis* is the most commonly detected NTM causing infection in humans and pigs (Cayrou *et al*., 2010). *Mycobacterium avium* subsp. *avium* occasionally infects pigs and humans but is generally regarded as an obligate pathogen of birds, causing contagious avian

tuberculosis (Cayrou *et al*., 2010). *Mycobacterium intracellulare* is an environmental organism associated with lymphadenopathy (enlarged lymph nodes) in children and progressive pulmonary disease in elderly women (Cayrou *et al*., 2010).

Saprophytic mycobacteria are the largest group found in the environment. Other names that have been used to define this group include atypical, "nontuberculous", environmental mycobacteria or Mycobacteria Other Than Tuberculosis (MOTTs) of which about one third have been associated with diseases in humans and animals. The MOTTs species that have been found to cause disease in human and animals are: *M. kansasii, M. paratuberculosis, M. scrofulaceum, M. simiae, M. habana, M. interjectum, M. xenopi, M. heckeshornense, M. szulgai, M. fortuitum, M. immunogenum, M. chelonae, M. marinum, M. genavense, M. haemophilum, M. celatum, M. conspicuum, M. malmoense, M. ulcerans, M. smegmatis, M. wolinskyi, M. goodii, M. thermoresistible, M. neoaurum, M. vaccae, M.palustre, M. elephantis, M. bohemicam and M. septicum* (Lavania *et al*., 2007).

Classification of the genus *Mycobacterium* can further be based on a polyphasic approach including phenotyping, chemo-taxonomy and molecular analysis, as well as the structure of the peptidoglycan (Prakash *et al*., 2007).

2.1.2 Cellular morphology and staining

Mycobacteria are obligate intracellular, non-motile, non-capsulating, non-spore forming rods measuring 0.2-0.6um by 1.0-10um with a slender, straight or slightly curved shape (Biet *et al*., 2005; Thoen *et al*., 2006). The cell wall of mycobacteria contains lipids which include mycolic acids, phosphatidyl inositol mannosides, phthiocerol dimycocerosates and lipoglycans which play important roles in maintaining integrity of the cell envelope (Jackson, 2014); modulating early immune responses of macrophages to infection (Arbues *et al*., 2014);

resistance to free radicals and inhibition of synthesis of anti-mycobacterial cytokines by the host immune system (Singh *et al*., 2016).

Mycobacterium are difficult to stain but once stained, they resist decolourization even by weak mineral acids such as 3% hydrochloric acid in ethanol (Quins .P. *et al*, 2013) hence the name acid fast bacilli (AFB). Acid fastness is due to the presence of unsaponifiable wax in the cell wall composing of mycolic acid and other lipids. Mycobacteria have a lipid rich, hydrophobic cell wall, which is substantially thicker than most other bacteria (Niederweis, 2008) that renders mycobacteria impermeable to hydrophilic nutrients and resistant to heavy metals, disinfectants, and antibiotics (Niederweis, 2008).

Mycobacteria are considered to be Gram positive bacilli based on cell wall structure though they cannot be stained by Gram stain (Quins .P. *et al*, 2013). Other genera with the same cell wall type, include *Corynebacterium, Gordonia, Nocardia, Rhodococcus* and *Tsukamurella* (Sokolovská *et al*., 2003). The analysis of mycolic acid content has been of great use in the classification of mycobacteria (Butler and Guthertz, 2001).

2.1.3 Cultural characteristics

Under laboratory conditions glycerol is the preferred carbon source for copious growth of many mycobacterial species (Keating Lisa A. *et al*., 2005), while *M. bovis*, *M. africanum* and *M. microti* are unable to use glycerol as a sole carbon source, but addition of pyruvate to glycerinated media enables growth (Keating Lisa A. *et al*., 2005). Addition of egg yolk to the mycobacteria growth medium was found to significantly enhance the rate of growth, due to the presence of phospholipids that are readily utilised by the mycobacteria to synthesize lipids required for their growth (Leisching *et al*., 2016). Later synthetic phospholipids in the form of polyoxyethylene sorbate compounds (Tweens) were introduced into growth media. Apart from enhancing growth, Tweens have been found to lower tendency of the

mycobacteria to aggregate, giving diffuse homogenous cultures. Tweens contain oleic acid that serves as a potent carbon and energy source (Leisching *et al*., 2016).

Mycobacteria are obligate aerobes which grow on complex organic media; which can be solid culture media which is either egg based such as Lowenstein-Jensen (L-J) containing malachite green to inhibit growth of contaminating bacteria and fungi; or agar based such as Middlebrook 7H10 and Middlebrook 7H11. Liquid media generally used is Mycobacteria Growth Indicator Tube (MGIT) such as Modified Middlebrook 7H9 broth. Complex media usually provide the full range of growth factors that may be required by bacterial pathogens and other fastidious bacteria whose nutritional requirement are complex (i.e., organisms that require a lot of growth factors, known or unknown). Colonies on egg based media (L-J) are small, rounded, moist, off-white (bluff) in colour, flat, wrinkled surface with irregular, thin margins and sparsely distributed (Pfyffer Gaby.E and Frantiska Palicova, 2007). Growth of mycobacteria in MGIT appears as suspensions colony-like clumps in the broth media.

Addition of pyruvate stimulates growth of *M. bovis* (OIE, 2009). Growth occurs within 3-6 weeks (up to 12 weeks) of incubation at 37^0C (OIE, 2009). Glycerol favours the growth of *M.tuberculosis* (Central TB Division and Directorate General of Health Services, 2009). Paranitrobenzoic acid (PNB) inhibits growth of the MTBC but allows other mycobacteria to grow (Giampaglia *et al*., 2007; Nepali *et al*., 2010). PNB has been successfully used by many researchers for *Mycobacterium* identification (Giampaglia *et al*., 2007; Nepali *et al*., 2010).

In the studies done by Giampaglia and Nepali showed that *Mycobacterium tuberculosis* does not grow in Lowenstein-Jensen (L-J) medium containing para-nitro benzoic acid (PNB); which can be a basis for its identification from other mycobacteria (Giampaglia *et al*., 2007; Nepali *et al*., 2010). MGIT has been used to isolate mycobacteria other than *M. tuberculosis*

complex (MOTTs) though many researchers have found high contamination rate, reducing it's sensitivity (Robbe-Austerman *et al*., 2013).

2.1.4 Environmental and chemical resistance

Mycobacterium can be found as natural inhabitants of the environment, found as saprophytes, commensals, and symbionts in the ecosystem (Wagner and Young, 2004). During hot weather for example can survive in feces for two months and in cold temperatures for up to six months. Survival will depend on environmental temperature and concentration of pathogen in the faeces (McCallan *et al*., 2014). The same author found out that *Mycobacterium bovis* can persist in slurry and in soil for almost 6 months. In developing countries manure is commonly used as a fertilizer in farms even before proper treatment (McCallan *et al*., 2014). Such manure can become a source of infection to animals through pasture and vegetation consumption (Ayele *et al*., 2004).

High temperatures and UV light will easily kill mycobacteria. Temperature of 65 °C or above for at least 30 minutes will destroy *Mycobacterium* (Pfyffer Gaby.E and Frantiska Palicova, 2007). Studies have shown that mycobacteria are resistant to freezing, dessication, acids, alkalis and some chemical disinfectants (Phillips *et al*., 2002). Mycobacterial cell wall has a high lipid and wax content which confers hydrophobicity, rendering these bacterial cells less susceptible to many chemical disinfectants including antibiotics (Russell, 2007). In general *Mycobacterium* cannot tolerate harsh environments such as prolonged exposure to heat, direct sunlight and dry conditions but can survive for long periods under dark, cold and moist conditions (Phillips *et al*., 2002). The waxy, hydrophobic, high lipid and impermeable cell wall of *Mycobacterium*, makes it resistant to substances such as acids, detergents, oxidative bursts, and antibiotics (Lambert, 2002).

Chemicals that have been shown to have bacteriostatic effect on mycobacteria includes: malachite green, quarternary ammonium compounds, hexachlorophene and chlorohexidine while formaldehyde vapour, chlorine compounds, 70% ethanol, hydrogen peroxide and 2% alkaline glutaraldehyde have bacteriocidal effect. Chemicals that are inactivated by the presence of organic matter such as alcohols cannot be used to disinfect protein containing matter such as mucous membranes (Russell, 2007).

2.2 Mycobacteriosis in cattle

2.2.1 Etiology

Mycobacteriosis in cattle is caused mainly by *Mycobacterium bovis* subsp.*bovis* but *Mycobacterium bovis* subsp.*caprae* which belong to the *Mycobacterium tuberculosis* complex (MTBC) group has also been detected (Aranaz *et al*., 2004; Bezos *et al*., 2012; Duarte *et al*., 2008; Shitaye *et al.*, 2006; Smith, 2003). The two subspecies also affect other domestic animals, wildlife and humans. Infections are commonly referred to as bovine tuberculosis (bTB) or more aptly as zoonotic tuberculosis. *Mycobacterium tuberculosis* has also been isolated in cattle (Ashford *et al*., 2001). *M. africanum* has been isolated from cattle meat carcasses in Ghana (Asante-Poku *et al*., 2014) and Nigeria (Asante-Poku *et al*., 2014).

Although not as widely as zoonotic tuberculosis, MOTTs infections have been reported in cattle. Studies done by Cleaveland *et al*.(2007) reported isolation of *Mycobacterium terrae, Mycobacterium avium, Mycobacterium chelonae, Mycobacterium gordonae, Mycobacterium fortuitum, Mycobacterium flavescens* and *Mycobacterium smegmati*as, as possible causes of mycobacteriosis in cattle (Cleaveland *et al*., 2007).

A retrospective study carried out in USA isolated fifty-five mycobacterial species in cattle; which included *M.bovis*, *M.avium* complex, *M. fortuitum/fortuitum* complex; *Mycobacterium shimoidei, Mycobacterium interjectum, Mycobacterium asiaticum, Mycobacterium szulgai*,

Mycobacterium kansaasii among others (Thacker *et al*., 2013); though animal infections by *M. kansasii* are very rare according to Gcebe and Hlokwe, (2017). A study done in Chad found *M. fortuitum* to be common in humans and cattle (Müller *et al*., 2008a). Another study in Uganda isolated MOTTs from animals exhibiting granulomatous lesions (Diguimbaye-Djaibé *et al.*, 2006; Oloya *et al*., 2007). In Kenya, MOTTs from tuberculous lesions in slaughter cattle were isolated, though their respective species were not characterised (Gathogo *et al*., 2012a).

Gcebe and Hlokwe, (2017) have reported isolation of MOTTs from different wildlife species and cattle. They isolated *M. asiaticum, M. avium, M. fortuitum, M. interjectum* among others. It should be noted that, isolation of MOTTs from an animal source does not necessarily imply an active disease status (Gcebe and Hlokwe, 2017).

2.2.2 Epidemiology of bovine mycobacteriosis

The epidemiology of mycobacteriosis has been well described by numerous authors in extensive detailed reviews from developed countries, where control and eradication programmes have been implemented for a long time (Tschopp *et al*., 2009); however in developing countries, the epidemiology is poorly studied and the epidemiological data is scanty (Tschopp *et al*., 2009). Information on risk factors of disease transmission to cattle, between cattle, cattle to humans and from human to human is mainly not available from the African context, with information available being extrapolated from experiences in the industrialised countries (Tschopp *et al*., 2009).

In Africa, comprehensive epidemiological studies have been done in Zambia (Munyeme *et al*., 2009), Tanzania (Katale *et al*., 2013), and Uganda (Oloya *et al*., 2007).

2.2.2.1 Geographical distribution

Mycobacteriosis caused by *Mycobacterium bovis* subsp.*bovis* has a worldwide distribution and according to information from OIE, 128 out of 155 countries reported the presence of mycobacteriosis in their cattle population between year 2000 and 2008. In developed world, control and elimination measures have been practiced for decades based on systematic test and slaughter of infected animals, meat inspection surveillance in abattoirs and milk pasteurization (EFSA and ECDPC, 2016; OIE, 2009).

In developing countries, such as Africa, Central and South America, parts of Asia and some middle East Countries; mycobacteriosis is still widely uncontrolled due to inability to support test-and-slaughter policies and the fact that it is viewed as a secondary disease compared with more readily transmissible diseases such as CBPP, FMD (OIE, 2009). Other impending factors in the developing world include: political instability, wars, insufficient collaboration within bordering countries, lack of quarantine, smuggling of animals across borders and lack or inadequate veterinary expertise. According to Cosivi *et al*., (1998) only seven nations in Africa consider mycobacteriosis as a notifiable disease and thereby apply control measures.

In Africa mycobacteriosis has been reported in domestic ruminants in Senegal, Burkina Faso, Mauritania, Ghana, Nigeria (Gathogo *et al*., 2012b), Chad (Diguimbaye-Djaibé *et al*., 2006), Mali (Müller *et al*., 2008a), Algeria (Sahraoui *et al*., 2009), Egypt (Mosaad *et al*., 2012) and Sudan (Asil *et al*., 2013) . Regionally the disease has been reported in Somalia and Ethiopia (Shitaye *et al*., 2007; Berg *et al*., 2009; Demelash *et al.*, 2009, Regassa *et al*., 2010); Tanzania (Kazwala *et al*., 2006); Burundi (OIE - World Organisation for Animal Health, 2012) and Uganda (Asiimwe *et al*., 2009).

In Kenya mycobacteriosis was first reported by Piers and Wright in 1946 who observed the presence of the disease in slaughtered bovine carcasses in selected districts. According to the

study, field veterinary staff were not taking a keen role in reporting the disease. Later the disease was detected in wild Olive Baboons around Maasai Game Reserve (Tarara *et al.*, 1985). Sapolsky and Else, (1987) reported mycobacteriosis in Baboons in Mtito Adei and implicated infected offals from a nearby slaughter house. Kang'ethe *et al.*,(2007) reported prevalence of mycobacteriosis in Dagoretti Division using Single Intradermal Tuberculin Test (SITT). Recently Gathogo *et al.*, (2012a) whose work focussed on *Mycobacterium bovis* subsp.*bovis* (zoonotic bovine tuberculosis) reported using lesions from bovine slaughtered carcasses in two main slaughter houses in Nairobi. Slaughter animals were from many parts of the country especially arid and semi-arid areas which supply bulk of the slaughtered animals into the city and its environs.

It is important to note that mycobacteriosis in cattle in Africa and Kenya included has concentrated on zoonotic bovine tercuolosis (caused by *Mycobacterium bovis* subsp.*bovis*). MOTTs are thought to be regionally distributed in the world, though literature is scanty (Gcebe and Hlokwe, 2017).

2.2.2.2 Prevalence of Mycobacteriosis

Global prevalence of human TB caused by *Mycobacterium bovis* subsp.*bovis* was estimated to be 3.1% of all human TB worldwide, accounting for 2.1% and 9.4% of pulmonary and extra-pulmonary respectively (H1 *et al.*, 2016). In cattle global prevalence has been estimated at 0.8% (Boukary *et al.*, 2011). In Europe and North America, 0.5% to 1% of human tuberculosis cases are estimated to be caused by *Mycobacterium bovis* subsp.*bovis* infection (Grange *et al.*, 1996). The low prevalence can be due to control and elimination measures that have been practiced for decades based on systematic test and slaughter of infected animals, meat inspection surveillance in abattoirs and milk pasteurization (OIE, 2009). Complete eradication of mycobacteriosis in developed countries has been hindered by the existence of reservoirs of the agent in wildlife species (OIE, 2009). Schiller *et al.*(2011) in their study

found a link between challenges in eradication of mycobacteriosis and the presence of the disease in wildlife populations. In developing countries where minimal or no control of mycobacteriosis in livestock, human TB cases due to *Mycobacterium bovis* subsp.*bovis* has been estimated at 10% to 15% (Ashford *et al*., 2001).

A study was conducted in two consecutive years of 2007-2008 in the Northern Ecuador to determine the prevalence of mycobacteriosis in dairy cattle using single and comparative intra-dermal skin test, reported animal prevalence of 7.4 % and 7.13%, and herd prevalence of 55% and 65% respectively for the two consecutive years (Proaño-Perez *et al*., 2006). In Pakistan, Peshawar district using Single Intra-dermal Tuberculin Test (SIDTT), reported 5% mycobacteriosis (Nawaz, 2013).

In Torodi region of Niger, a study done to determine prevalence of mycobacteriosis using comparative intra-dermal skin test in cattle herds under rural livestock production system reported 3.6% prevalence (Boukary *et al*., 2011), and in Niamey post-mortem lesions were used from an abattoir, which reported 0.19% of the cattle carcasses examined had lesions typical of mycobacteriosis (Boukary *et al*., 2012). Asil *et al*.(2013) reported 0.18% prevalence of tuberculosis in slaughterhouse cattle using tuberculous lesions in Sudan (Asil *et al*., 2013). An abattoir study to determine prevalence of mycobacteriosis in six cattle producing states of Northern Nigeria between year 2000 and 2004 found a prevalence of 4.05% where 302,700 cattle carcasses were sampled during the study period (Aliyu *et al*., 2009). A retrospective study conducted to generate epidemiological data on TB in cattle and humans in Enugu State, Nigeria through a survey in abattoirs and hospital, recorded 14.9% gross tuberculous lesions and overall prevalence of 1.4% (Nwanta *et al*., 2011). An abattoir surveillance study in El-Basateen abattoir, Cairo, Egypt between December 2010 and March 2011 showed 0.21% of the sampled carcasses had tuberculous lesions (Ramadan *et al*., 2012).

In Tanzania several studies have been carried out to determine prevalence of mycobacteriosis in different regions and production systems. Shirima *et al*.(2003) compared the prevalence of mycobacteriosis in pastoral and intensive production systems in the Eastern Tanzania using single intra-dermal tuberculin skin test, reported 2% and 1% for the intensive and pastoral production system respectively. In Northern Tanzania Cleaveland *et al*.(2007), reported mycobacteriosis prevalence of 0.9% and a herd prevalence of 11.8%. Swai and Schoonman, (2012) carried out a study in the Tanga region of North Eastern Tanzania where a smallholder representing intensive production system and traditional managed representing extensive production system, reported an individual animal and herd prevalence of 2% and 5.7% respectively.

Among the African countries more work has been carried out in Ethiopia by different researchers and in different times and regions. Demelash *et al*.(2009) used slaughterhouse lesions from five abattoirs in Ethiopia over a period of seven months between July 2006 and January 2007 which reported 23.9% tuberculous lesions in meat carcasses and prevalence of 10.2%. A cross-sectional study conducted in four Districts, Arsi region, Ethiopia, between October 2011 and March 2012, using milk samples from 13 cooperatives reported an overall bovine tuberculosis prevalence of 0.3% (Tschopp *et al*., 2010). Another study at Gondar Elfora abattoir from December, 2005 to June, 2006, also in Ethiopia reported 15.9% gross tuberculous lesions and overall mycobacteriosis prevalence of 2.9% (Worku and Abreham, 2016).

A study conducted to estimate the prevalence of bTB in cattle using gross examination of granulomatous lesions, identify mycobacteria species in suspected samples, and evaluate the economic impact of meat condemnation based on bTB-like lesions in the meat industry in Kigali, Rwanda; reported 0.9 % *M. bovis* (Habarugira *et al*., 2014). Another study conducted

in Mubende District, Uganda, using tubercuous lesions of slaughter cattle recorded prevalence of 9.7% (Nalapa *et al*.,2017).

Studies on isolation of MOTTs from bovine tissue sources in Africa have mainly focussed on either cattle from slaughter houses or on MOTTs that were coincidentally isolated from animal lesions while looking for *Mycobacterium bovis* (Cosivi *et al*., 1995). Two studies done in Chad and Uganda isolated MOTTs from more than 40% of the animals exhibiting granulomatous lesions (Diguimbaye-Djaibé *et al*., 2006; Oloya *et al*., 2007). Another study done in Chad found *Mycobacterium nonchromogenicum*, *Mycobacterium Avian* Complex (MAC) and *Mycobacterium fortuitum* were found to be common in humans and cattle (Diguimbaye-Djaibé *et al*., 2006), which reported prevalence of 7.3% from TB-like lesions on cattle meat carcasses. In Uganda, Kankya *et al*.(2011) isolated MOTTs in 15.5% of environmental samples by identifying 15 MOTTs species from 48 samples that included *Mycobacterium fortuitum* complex and *Mycobacterium avium* complex among others. In Kenya, Gathogo *et al*.(2012) reported mycobacteriosis prevalence proportion of 18.95% using tuberculous lesions from bovine slaughtered carcasses in two main slaughter houses in Nairobi. The same study isolated 24.4% MOTTs but were not characterised.

2.2.2.3 Transmission and risk factors of Mycobacteriosis

It has been confirmed by researchers that infectious animals shed *Mycobacterium* via milk, saliva, feces/urine and discharging lesions (Phillips *et al*., 2003). The main route of infection in cattle is mainly through aerosol exposure, facilitated by close contact between animals (Neill *et al*., 2005). This fact has been supported by high frequency of tuberculous lesions found in the respiratory tract and associated lymph nodes in cattle (Johnson *et al*., 2007). Ingestion of contaminated products (such as pasture and water) is generally considered to be a secondary, less important route of transmission (Menzies and Neill, 2000). Vijay Chandra JHA *et al*., (2007) isolated *Mycobacterium bovis* in milk and feces from milking buffaloes

and cattle in Nepal. This indicates that transmission to young animals by milk should not be ignored.

Recent publications from Africa suggest that ingestion of *Mycobacterium* might be an important mode of disease transmission in cattle, since mesenteric lymph nodes were shown to be more affected than mediastinal lymph nodes (Ameni *et al*., 2003; Cleaveland *et al*., 2007). Other rare routes of infection include cutaneous, genital during coitus, congenital through the umbilical cord, pseudovertical transmission due to udder infections (Menzies and Neill, 2000). Shirima *et al*.(2003) observed that introduction of exotic breeds of cattle in Africa in the last century from Europe led to intensive livestock farming (locally known as zero grazing) which has promoted close contact between animals favouring mycobacteriosis transmission. Extensive livestock farming which is common in arid and semi-arid areas of Africa also encourages close contact of animals while grazing communally, at water points, vaccination sites, at livestock markets and during transportation. This coupled with survival of mycobacteria for some time in environment such as in buildings, transport vehicles, on pasture, in slurry, and in manure encourages transmission of mycobacteriosis among animals (Ayele *et al*., 2004) .

Mycobacterium could be transmitted to humans from cattle through the inhalation of cough sprayed from infected cattle, or from handling or consumption of milk contaminated with the mycobacteria (Wedlock *et al*., 2002). A regular high prevalence of mycobacteriosis in cattle can be correlated with the isolation of *Mycobacterium* in milk samples; this is demonstrated by Vekemans *et al*., (1999) who reported isolation of mycobacteria in 26% of 60 retailed milk samples collected from markets in Burkina Faso. A study done in Nigeria by Ibrahim *et al*., (2012) estimated that in Africa 90% of milk is consumed either as raw or fermented thus increasing the risk of mycobacteriosis transmission from animals to humans due to lack of formal treatment. Eating of raw or undercooked meat can also infect humans (Cosivi *et al*.,

1998). Human infection by *Mycobacterium* has been reported due to inhalation of infected droplets released by infected animals or because of contact with infected droplets through mucous membranes and broken skin associated with people that are frequently in contact with animals such as slaughter house workers, farmers, vets, milkers etc (Ameni *et al*., 2003; de la Rua-Domenech, 2006; Moda *et al*., 1996; Une and Mori, 2007). Ameni *et al*.(2003) showed that 99% of mycobacteriosis cases were caused by raw meat or undercooked meat consumption versus 1% of undercooked meat consumption in Central Ethiopia. The same study showed herd and individual animal prevalences of 42.6% and 7.9%, respectively. Also this study showed that 24.5% of the interviewed households had experienced at least one human TB case in the family. Trans-cutaneous transmission has been reported when performing post-mortem on infected carcasses (Thoen *et al*., 2006).

Though a rare occurrence type of transmission; studies have revealed that infected human beings shed the infection through urine which if it comes into contact with pasture or vegetation it will contaminate it and animals while craving for salt lick leading to infection (Ayele *et al*., 2004; Collins and Grange, 1983). Evans *et al*., (2007), reported cases of patients with pulmonary mycobacteriosis transmitting the disease to animals, especially from immunosuppressed patients mainly due to HIV infections (Evans *et al*., 2007).

Sunder *et al*.(2009) reported a case in France where a man was diagnosed with pulmonary mycobacteriosis infection after having worked in a slaughter house for 7 years. The victim was found to have infected his daughter who previously did not have had any contact with cattle herds or taken any untreated animal products. It was thus deduced that the only probable source of infection to the woman was most probably the infected father. Figure 1 depicts the transmission cycle of *Mycobacterium* between cattle and humans, with the thickness of the arrows suggesting higher probability of transmission.
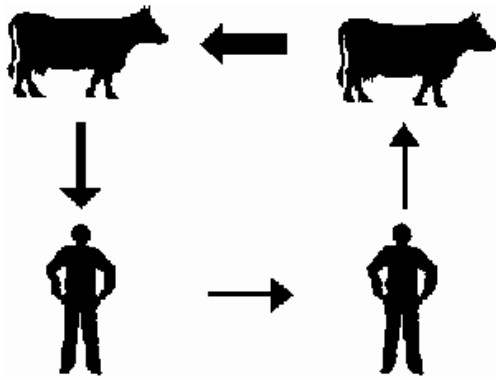
Figure 2: Cycle of Mycobacterium transmission between cattle and humans. The thickness of the arrows suggests higher probability.

MOTTS are found in soil, dust, food, water (fresh and sea), animals, plant material, and birds. Most infections appear to be acquired by ingestion, aspiration, or inoculation of the organisms from these natural sources; however the specific source of individual infections is usually not easily identified. Evidence from literature of animal-to-human or human-to-human transmission of MOTTs is scanty. Transmission routes between animals are directly associated with the localization of granulomas, with pulmonary lesions being indicative of airborne transmission, whereas mesenteric lymph-node lesions showing alimentary transmission. Vertical transmission through placental or umbilical infection has also been implicated (Thoen *et al.*, 2009), with urogenital route of MOTTs being suspected (Shrivastava *et al*., 2014).

Since their clinical relevance was unknown, MOTTs have been neglected for many years as they have always been recognized as environmental contaminants (Covert *et al*., 1999). Most infections appear to be acquired by aspiration, ingestion, or inoculation of the organisms from these natural sources; however the specific source of individual infections is usually not identified.

Various studies in Africa have identified various risk factors for mycobacteriosis transmission at different spatial levels. At individual animal level, as shown by several cross-

sectional studies carried out in Tanzania, Zambia and Chad (Cleaveland *et al*., 2007; Cook *et al*., 1996; Müller *et al*., 2008; Inangolet *et al*., 2008). Males were more significantly more affected by bTB than female animals (Kazwala *et al*., 2001). Bos indicus (zebu) have been found to be more resistant to bTB than Bos Taurus (Exotic breeds), where imported dairy animals are generally kept under intensive management conditions (Firdessa *et al*., 2012).

At the herd level; herd size increased infection due to animal density which increased exposure (Costello *et al*., 1998), type of production system, where dairy cows were more susceptible due to production stress and the life expectancy of dairy cattle being longer than beef cattle, dairy tend to be kept in intensive production system raising stress and increasing contact between animals; while beef are more kept in extensive system (Cosivi *et al*., 1998).

In Tanzania, a high prevalence was reported in pastoral cattle with high numbers of cattle kept under intensive production system (Katale *et al*., 2013), and in Uganda, the prevalence bovine mycobacteriosis was higher in agro-pastoral than in pastoral production systems, probably because of the closer contact between cattle and the more humid conditions in agro-pastoral production systems (Oloya *et al*., 2007). Introduction of infected animals into the herd could increase mycobacteriosis transmission (Humblet *et al*., 2009). Movement of livestock to follow grazing and water resources in pastoral areas may increase animal to animal contact favouring transmission of bovine mycobacteriosis (Dejene *et al*., 2016).

Mycobacterium bovis has the widest host range of all pathogens, combined with complex epidemiological pattern, that involve interaction of infection among human, domestic animals and wild animals (Gemechu *et al.*, 2013). Infection is influenced by factors associated to host, environment and the pathogen itself (Ameni *et al*., 2011). Husbandry practices such as close animal contact, high stocking rate and overcrowding raise infection rate (Katale *et al*., 2013).

Various studies have shown that *Mycobacterium* is maintained in the environment by wild hosts that include kudu and African buffalo in southern Africa. Species reported to be spill-

over hosts include sheep, goats, horses, pigs, dogs, cats, ferrets, camels, many species of wild ruminants including deer, elephants, rhinoceroses, foxes, coyotes, mink, primates, hares, raccoons, bears, warthogs, large cats (including lions, tigers, leopards, cheetahs and lynx) and several species of rodents (OIE, 2009). Most mammals may be susceptible therefore the above listed may not be exhaustive (OIE, 2009; Falkinham, n.d.).

Mycobacteria Other than Tuberculosis (MOTTs), previously non-tuberculous mycobacteria (NTM), or environmental mycobacteria (EM) are believed to be natural inhabitants of the environment, found as saprophytes, commensals, and symbionts in the ecosystem. The majority of NTM are opportunistic pathogens, and true inhabitants of the environment found in soil, dust, food, water (fresh and sea) as saprophytes, commensals and symbionts (Falkinham, n.d.), in ecosystems shared between humans and animals (Falkinham, n.d.). Soil and natural open water sources are known to contain mycobacteria, and play a key role as sources for human and animal infections, especially if contaminated with animal wastes from domestic and even from wildlife (van Ingen *et al*., 2009). In Africa, high interaction between wild animals, the environment, and humans seem to be very common posing a risk of MOTTs contamination of natural waters and transmission between animals and humans (Katale *et al*., 2014).

2.3 Pathogenesis

Inhaled bacilli are phagocytosed by alveolar macrophages that may either clear the infection or allow the mycobacteria to proliferate. In the latter instance, a primary focus may form, mediated by cytokines associated with a hypersensitivity reaction that consists of dead and degenerate macrophages surrounded by epithelioid cells, granulocytes, lymphocytes, and later, multinucleated giant cells (Flynn *et al*., 2011). The purulent to caseous, necrotic center may calcify, and the lesion may become surrounded by granulation tissue and a fibrous capsule to form the classic "tubercle" (Quins .P. *et al*, 2013). The primary focus plus similar

lesions formed in the regional lymph node is known as the "primary complex." In alimentary forms of disease, the primary focus may be found in the pharynx or mesenteric lymph nodes or, less commonly, in the tonsils or intestines (Quins .P. *et al*, 2013).

The primary complex seldom heals in animals and may progress slowly or rapidly. Dissemination through vascular and lymphatic channels may be generalized and rapidly fatal, as in acute miliary TB. Nodular lesions may form in many organs, including the pleura, peritoneum, liver, kidney, spleen, skeleton, mammary glands, reproductive tract, and CNS (Flynn *et al*., 2011).

In cattle *Mycobacterium* lesion is composed of a central caseous necrosis, sometimes with calcification and surrounded by epithelial cells, multinucleated giant cells, lymphocytes and neutrophils (Neill *et al*., 1994). Location of these lesions in cattle is mainly the lymphatic tissues of the thoracic cavity which comprise of bronchial and mediastinal lymph nodes; the head region which is served by the parotid, retropharyngeal and sub-maxillary lymph nodes. Lesions in mesenteric lymph nodes is uncommon (Neill *et al*., 1994).

2.4 Clinical signs of Mycobacteriosis in cattle

Mycobacteriosis is a chronic debilitating disease in cattle. Starts as asymptomatic, however, in late stage, there is progressive emaciation, mild fluctuating fever, weakness and in-appetence (Thoen *et al*., 2016). When infection reaches the lung, dyspnoea, moist cough or trachypnoea due to bronchopneumonia occurs. Cough occurs only once or twice at a time and is low suppressed and moist which is easily stimulated by squeezing the pharynx or by exercise and is most common in morning and in cold weather (Thoen *et al*., 2016). In the terminal stage, the animal becomes extremely emaciated and may develop acute respiratory distress. Involvement of respiratory tract and its role in pathogenesis of disease is evidenced by the predominant distribution of lesions in the upper respiratory tract, lungs and lymph nodes of the head region. In the miliary form of the disease, there is progressive emaciation,

in spite of the animal maintaining good appetite (Thoen *et al*., 2016). Infection of the udder has been found in varying proportions of tuberculous animals, from 1-2%, up to 5.4% (Cosivi *et al*., 1998).

2.5 Pathology

Mycobacteriosis causes a chronic granulomatous, caseous-necrotising inflammation in lungs, associated lymph nodes (Domingo *et al*., 2014), mediastinal, retropharyngeal, parotid, submaxillary and portal lymph nodes along with tissues affected. Liver, spleen and the surfaces of body cavities may be affected. Pathology of tuberculosis is characterized by the formation of granulomatous lesions, which can within the course of the disease regress or exhibit extensive necrosis, calcify or liquefy and subsequently lead to cavity formation (Admassu *et al*., 2015). Lesion necrosis, liquefaction, mineralization and regression represent some of the outcomes of these interactions that dictate lesion size and appearance and ultimately the presentation of disease in the host (Waters and Palmer, 2015). In chronic or latent infection, this type of granuloma can become calcified, with the calcification process beginning within the caseous center. A calcified granuloma generally represents a successful immune response and is associated with fewer inflammatory cells than other granulomas (Flynn *et al*., 2011).

2.6 Public health importance, control and prevention of mycobacteriosis

Mycobacteriosis caused by *Mycobacterium bovis* subsp.*bovis* is an important public health concern worldwide and especially in developing countries where preventive measures are inadequate or lacking (Moda *et al*., 1996). This fact makes zoonotic tuberculosis a worldwide problem requiring intensive control and preventive measures for its eradication. In developed countries, human infections have drastically been reduced by eradicating the disease in cattle through high standards food safety measures such as pasteurization of milk, test-and-slaughter method, and slaughter house surveillance by detection of affected carcasses and

destruction of those affected (EFSA and ECDPC, 2016). In these countries contribution of *Mycobacterium bovis* to all Tb cases in humans has been estimated to be for example, less than 2% in USA, less than 1.5% in UK and Ireland (de la Rua-Domenech, 2006) and below 1.4% in Netherlands (Mirsaeidi *et al*., 2014).

In developing countries contribution of *Mycobacterium bovis* subsp.*bovis* to TB incidences in human has not been determined accurately due to such factors as under-reporting, inefficiency in diagnosis due to diagnostic limitations of many laboratories, consumption of untreated or inadequate treatment of animal products, high prevalence of HIV/AIDS cases causing immunosuppression making the infected vulnerable to *Mycobacterium bovis* subsp.*bovis* and other opportunistic infections (Grange, 2001).

Zoonotic TB in cattle is mainly caused by *Mycobacterium bovis* subsp.*bovis* and can be acquired by humans through the inhalation of cough droplets sprayed from infected cattle, or from handling or consumption of milk contaminated with the microorganisms (Wedlock *et al*., 2002). Eating of raw or undercooked meat can also infect humans. Signs in human, include fever, night sweats, weight loss, cough if lungs are involved, abdominal pain and diarrhea if digestive system is affected.

Because of local habits concomitant with a high prevalence of mycobacteriosis in cattle, some countries present a real zoonotic risk of mycobacteriosis transmission. Ameni *et al*., (2003) showed that 99% of cases were caused by raw meat or undercooked meat consumption versus 1% of only undercooked meat consumption in Central Ethiopia. This study established that 24.5% of the interviewed households had experienced at least one human TB case in the family.

Active competition in all Sub-Saharan African countries, between large-scale commercial food enterprises and smaller less-regulated farmers who frequently ignore safety standards

for hygiene and product quality, further increases the risk of the zoonotic TB (Cosivi *et al*., 1998). These smaller farmers sell directly to final consumers and contribute to the spread of mycobacteriosis. Furthermore, 90% of the total milk produced by these countries and consumed by people is either unpasteurized or fermented, without pasteurization. In Kenya 88% of milk is marketed informally without recommended treatment (Wambugu *et al*., 1998).

According to Office International des Epizooties (OIE, 2009), the standard control measure applied to mycobacteriosis is test and slaughter. Mycobacteriosis eradication programs consisting of post-mortem meat inspection, intensive surveillance including on-farm visits, systematic individual testing of cattle and removal of infected and in-contact animals as well as movement controls have been very successful in reducing or eliminating the disease (Etter *et al*., 2006).

Post mortem meat inspection of animals looks for the tubercles in the lungs and lymph nodes (OIE, 2009). Detecting these infected animals prevents unsafe meat from entering the food chain and allows veterinary services to trace-back to the herd of origin of the infected animal which can then be tested and eliminated if needed. Pasteurization of milk of infected animals to a temperature sufficient to kill the bacteria has prevented the spread of disease in humans (Cosivi *et al*., 1998).

MOTTs have been found to infect animals and humans, causing lesions and symptoms similar to those caused by obligatory pathogenic mycobacteria, implying their potential role as pathogens (Mirsaeidi *et al*., 2014). However, reports of animal diseases caused by MOTTs are scanty. Studies on tuberculosis in animals have concentrated mainly on zoonotic bovine tuberculosis, neglecting the potential pathogenic and zoonotic role of other types of mycobacteria. Exposure to MOTTs has different public health implications, in humans they

are capable of causing pulmonary disease, disseminated disease or localized lesions in both immunocompetent and immunocompromised individuals (Kankya *et al*., 2011).

Mycobacterium fortuitum complex, saprophytic species present in various habitats such as lakes, surface water, potable water and soil and can colonise healthy animals and humans without causing disease. In animals, has been isolated in unpasteurised milk, in the environment of pig farms, been detected in dogs and cats with diarrhoea, from the lymph nodes of slaughtered cattle (Bercovier and Vincent, 2001). In human *M.fortuitum* has been associated with cutaneous and deeper infections after trauma, pulmonary disease and corneal infections (Kankya *et al*., 2011). Post trauma infections suggest the mycobacteria is widespread in the environment. Other syndromes in human include osteomyelitis, joint infections, lymphadenitis and endocarditis. Most of these syndromes have been reported in immunosuppressed and especially due to HIV/AIDS (Kankya *et al*., 2011).

Mycobacterium celatum, has been reported in domestic ferret (*Mustela putorius furo*) (Piseddu et al., 2011) and one in a white- tailed trogon (*trogon viridis*)(Bertelsen *et al*., 2006). Infection in animals range from classical tuberculous lesions with well-defined granulomas to poorly defined granulomas (Bertelsen *et al*., 2006). In human *M. celatum* has been recognised as a causative agent of pulmonary and disseminated infections in immunosuppressed humans (Lai *et al*., 2010, p.), especially with pre-existing lung infection.

Mycobacterium shimoidei also an opportunistic pathogen, has been previously isolated only among subjects with pre-existing lung diseases such as emphysema, previous tuberculosis, silicosis, lung carcinoma, (Tortoli *et al*., 1999) and severely immunocompromised (Galizzi *et al*., 2013). *Mycobacterium shimoidei*, was first isolated from a respiratory infection in a Japanese patient in 1975.

Mycobacterium interjectum is a rare and newly described cause of human infection. It was first described in 1993, causing cervical lymphadenitis in an 18-month-old German boy

(Springer *et al*., 1993). It was subsequently reported in 9 pediatric cases of necrotizing lymphadenitis (Mirant-Borde *et al*., 2013). It has also been isolated in the sputum of patients with chronic obstructive lung disease or HIV infection, in the urine of an asymptomatic elderly female, and in the stool of an AIDS patient with diarrhea (Tortoli *et al*., 1999). *Mycobacterium interjectum* has been well documented as a source of infection in children and immunocompromised adults; however, there are no descriptions of disease in healthy individuals.

Mycobacterium szulgai, is a relatively uncommon MOTT, accounting for less than 1% of all MOTTs isolations. *M szulgai* was first isolated in humans in 1972 (Kim *et al*., 2014). Diseases caused are pulmonary, cervical lymphadenopathy, carpal tunnel syndrome, cutaneous infections, osteomyelitis and less common disseminated diseases (Kang-Birken and Prichard, 2006).

Some MOTTs species, like *Mycobacterium kansasii, Mycobacterium marinum*, and *M. fortuitum* have been implicated to induce non-specific immune response leading to false positive reactions in tuberculin testing, thus interfering with the diagnosis of bovine tuberculosis either by tuberculin intra-dermal test or gamma interferon tests (Thacker *et al*., 2013).

2.7 Diagnosis of Mycobacteriosis

Mycobacteriosis can be diagnosed in live animals (OIE, 2009) and also during post-mortem examination of slaughtered carcasses (Corner, 1994; OIE, 2009). In live animals; clinical signs, tuberculin skin test and gamma interferon assay (Bovigam[R]) can be used. At post-mortem, pathological lesions and acid fast staining are preliminary tests while culture and DNA analysis are confirmatory.

2.7.1 Live animals

2.7.1.1 Clinical signs

Clinical diagnosis of mycobacteriosis in cattle is difficult due to the chronic nature of the disease and the wide variety of symptoms depending on the location of infection. The disease is progressive with two main stages: Initial phase involves TB granuloma (tubercle) formation which occurs at the site of infection and the local lymph node. This phase is asymptomatic ((European Food Safety Authority (EFSA), 2004).

Cattle with miliary tuberculosis in which infection is spread throughout the body show progressive emaciation, high appetite, fluctuating temperature, chronic and moist cough due to bronchopneumonia, dysphagia (difficult swallowing), noisy breathing due to swollen lymph nodes pressing the surrounding organs (Radostits C.M., 2007; European Food Safety Authority (EFSA), 2004). Enlargement of supramammary lymph nodes may result to mastitic TB (European Food Safety Authority (EFSA), 2004). It is difficult to diagnose mycobacteriosis using clinical signs only due to similarities in clinical manifestations with diseases such as CBPP, Pasteurella, Corynebacterium pyogenes pneumonia, traumatic pericarditis, liver fluke infestation (Grist, 2009).

2.7.1.2 Tuberculin Skin Test

This test measures the delayed type hypersensitivity response after injecting the cow intradermally with tuberculin. The form being used presently is purified protein derivative (PPD) which can either be of bovine origin (PPD-B) or of avian origin (PPD-A) or both. PPD is a crude extract of water soluble protein from a heated culture of *Mycobacterium bovis* (PPD-B) or *Mycobacterium avian* (PPD-A). The PPD-B can be used alone and the test is called single intradermal skin sensitivity test (SISST) or PPD-B and PPD-A together and the test is called comparative intradermal skin sensitivity test (CISST). Tuberculin skin test is the

standard procedure available internationally and recommended by OIE for the diagnosis of bovine TB in live animals (OIE, 2009).

In bovine, tuberculin is injected at the neck region which is much easier to measure skin thickness with calipers. In comparative sensitivity test PPD-B and PPD-A are injected side by side around 12cm apart and skin swelling is measured after 72hrs. According to OIE standard procedures the test result is considered positive, if the difference in the increase of skin thickness at the site of PPD-B injection is more than 4mm greater than the increase in skin thickness at the site of PPD-A injection (OIE, 2009). The result is inconclusive if the difference in skin thickness is between 2–4mm, requiring repeat after 42 days and negative if the difference is below 2mm (OIE, 2009). The sensitivity of the single intradermal comparative cervical test (SICCT) in GB was recently estimated at 81-85%, while the specificity of the test was estimated in excess of 99.9% (Rhodes *et al*.,2000).

Bovine tuberculosis diagnosed in the field using Purified Protein Derivative (PPD) antigen in live cattle is perceived to be the first line diagnostic tool. However, this test lacks specificity due to antigenic similarity among various members of the *Mycobacterium tuberculosis* complex (MTBC) and also with other mycobacterial species including Mycobacterium Other Than Tuberculosis (MOTTs). Besides being opportunistic pathogens, some MOTTs species may colonize the host without development of any disease, but affects immune system thus hampering the immuno-diagnosis of bovine tuberculosis by tuberculin based assays (Gcebe and Hlokwe, 2017).

2.7.1.3 Gamma interferon assays (BovigamR)

The gamma interferon assay (IFNγ) blood test for bTB was developed in Australia. The test is an in vitro assay based on detection of specific cell mediated immune response by circulating lymphocytes. It involves the stimulation of heparinized whole blood *in vitro* with PPD-B and

PPD-A (Purified Protein Derivative either of Bovine or Avian origin) to produce lymphokine gamma interferon (IFNγ). This test makes use of the comparison of gamma interferon production following stimulation with PPD-B and PPD-A. Detection of IFNγ is carried out using a sandwich ELISA after incubation of about 16-24 hrs with a specific antigen. The test uses two monoclonal antibodies to bovine gamma-interferon.

The IFNγ test is marginally more sensitive than the skin intra-dermal test and, crucially, it can detect infected animals that are negative to the tuberculin skin test. Several studies have shown that more infected cattle could be identified by using both the tuberculin skin test and the IFNγ test in parallel leading to detection and early removal of animals that were positive to either test (Clegg *et al*., 2017). The sensitivity of the IFNγ test can be high, around 90%, and its specificity, estimated at 96.7% in GB (Rhodes *et al*.,2000). The advantage of this test is that infected animals are detected early and only one visit to the farm is required to take blood unlike tuberculin intra-dermal skin test which requires revisit. Gamma interferon assays only detetects zoonotic tuberculosis.

2.7.2 Post-Mortem diagnosis of Mycobacteriosis

2.7.2.1 Detection of tuberculous lesions

Sensitivity of post-mortem procedures to detect mycobacteriosis is generally affected by meat inspector's work load, time and diligence (Corner *et al*., 1990). Detection of mycobacteriosis using pathological lesions involves visual observation, palpation and incision. Incision involves cutting of surfaces and examining them under bright light for the presence of any abscess, cheesy mass, and tubercles (Corner, 1994). Studies have shown that careful examination of as few as 6 pairs of lymph nodes especially of the thoracic region, lungs and the mesenteric lymph nodes can result to 95% detection of mycobacteriosis in bovine carcasses (Corner, 1994). At necropsy, tubercles are most frequently seen in bronchial,

mediastinal, retropharyngeal and portal lymph nodes and may be the only tissue affected (Tintagu Gizaw, 2017).

A presumptive diagnosis of bovine tuberculosis can be made following the macroscopic detection of these granulomatous lesions in different organs of infected animals (Ramos *et al*., 2015). Macroscopically, a tuberculous granuloma usually has a yellowish appearance and is caseous, caseo-calcareous, or calcified in consistency. Occasionally, its appearance may be more purulent in cervids and camelids (OIE, 2009).

Detection of mycobacteriosis in most African countries is based on the post-mortem findings of tuberculous lesions (Shitaye *et al*., 2006). Meat inspection provides very significant insight into the prevalence of many animal diseases, mycobacteriosis included (Grist, 2013). However diagnosis based on post-mortem examination at meat inspection may not be enough but preliminary before other confirmatory tests. Challenges that can be encountered are the inability to differentiate mycobacteriosis from other non-tuberculous lesions such as parasitic granulomas, bacterial abscesses, bacterial or mycotic pyogranulomas which may be difficult to differentiate macrospically (Liebana et al., 2008; OIE, 2009). There are other pathogens like *Actynomices bovis* and *Trueperella pyogenes* which produce granulomatous inflammation and morphologic characteristics similar to that produced by *Mycobacterium* (Biet *et al*., 2005).

In summary routine abattoir inspection is less effective, with low sensitivity due to: lack of regular competency testing of meat inspectors, heavy workloads with short time spent per carcass and sometimes poor physical facilities at abattoirs. The conventional post mortem examination has detected approximately 47% of presumptive bTB lesions in carcasses of cattle slaughtered (Biet *et al*., 2005).

2.7.2.2 Acid fast staining technique

The fastest, simplest and preliminary method to confirm a mycobacteria infection is a direct acid fast bacilli (AFB) smear examination from a suspect animal lesion sample. Ziehl-Neelsen (ZN) stain work on the simple principal that any mycobacteria present in the smear retain an arylmethane dye such as carbol fuschin within the cell giving an acid fast reaction. The method was first developed by Paul Erhlich and improved later by Ziehl and Neelsen. *Mycobacterium* are difficult to stain but once stained, they resist decolourization even by weak mineral acids such as 3% hydrochloric acid in ethanol (Quins .P. *et al,* 2013) or 25% sulphuric acid. Therefore they are referred to as acid fast bacilli (AFB). Acid fastness is due to the presence of unsaponifiable wax in the cell wall composing of mycolic acid and other lipids.

2.7.2.3 Culture

In veterinary laboratories culture is considered the ''gold standard'' for detection of mycobacteria (Richter *et al*., 2003; Collins, 2011). Solid media used can either be egg-based such as Loweinstein-Jensen(L-J) or Stonebrink Leslie solid culture media (Gangadharam and Jenkins, 1998). Agar-based culture media such as Middlebrook 7H10, 7H11 can also be used. Most mycobacteria require atmospheric oxygen for growth but some such as *Mycobacterium bovis* are microaerophilic (Grange *et al*., 1996).

Solid culture media can provide information about the species of mycobacterial growth without carrying out any biochemical or molecular tests. Liquid cultures such as BACTEC 460, MGIT (Mycobacterial Growth Indicator Tube) which have enriched Middlebrook 7Ha with antibiotics and growth promoters added. MGIT liquid media is fully automated and can monitor the growth of mycobacteria by use of oxygen quenching fluorescent sensor (OIE, 2009). Advantage of liquid media is that microorganisms can easily move within media to get

nutrients while in solid media movement is limited. Disadvantage of liquid media is that it is expensive and requires special equipment.

Samples for culture are first decontaminated to inactivate other bacteria that might be present in the sample to avoid their faster growth and media nutrient exhaustion which can render *Mycobacterium bovis* growth unfeasible. Solid media is prepared as slants in screw-capped bottles with malachite green dye (0.025g/100ml) being used as a selective agent. Growth of *Mycobacterium bovis* is slow taking up to 8weeks (averagely 6-8 weeks). Colonies grow on Loweinstein-Jensen media as small, moist, smooth, rounded with irregular edges (Murray, Patrick R, 1995) with off-white or bluff colour which breaks easily (OIE, 2009).

Reasons for not recovering mycobacteria from pathologic specimens during culturing may include: extended period (time) between collection of specimens and submission to the laboratory for analysis (Bolaños *et al*., 2017), tubercle bacilli could die as part of the tissue necrotic process in calcified and caseous necrotic granulomas (Demelash *et al*., 2009), the possibility that certain proportion of mycobacterial organisms may be killed by disinfection and decontamination methods used (Milian-Suazo *et al*., 2000), carcasses may be infected with mycobacteria other than *M. bovis* that would not replicate in the *M. bovis*-selective culture medium (Milian-Suazo *et al*., 2000), or other pathogens with different culture growth requirements (Müller *et al*., 2008a) and granulomatous lesions can be caused by other infectious agents such as fungi, *Staphylococcus*, *Actinomyces*, and *Actinobacillus* spp. and other foreign bodies (Demelash *et al.*, 2009).

2.7.3 Molecular Diagnosis of Mycobacteriosis

Presently different molecular tools have been developed to isolate and differentiate between *Mycobacterium* isolates (Oloya *et al*., 2007). Polymerase Chain Reaction principal is applied in these methods. PCR technique involves detection of the genetical material that is unique

and specific to an organism of interest (OIE, 2009). Different molecular tools have been developed to differentiate between mycobacterial isolates. Notable are spoligotyping and Restriction Fragment Length Polymorphism (RFLP) based molecular techniques (Haddad .N *et al.*, 2004). Spoligotyping is designed to detect the presence or absence of unique spacers within the direct repeat (DR) locus of the *Mycobacterium bovis* genome. It is known to distinguish between phenotypically different strains and has been successfully used to type *M. bovis* isolates from different African countries such as Nigeria (Cadmus *et al.*, 2006), Chad (Diguimbaye-Djaibé *et al.*, 2006), Cameroon (Njanpop-Lafourcade *et al.*, 2001), Madagascar (Rasolofo Razanamparany *et al.*, 2006) and Tanzania (Kazwala *et al.*, 2006).

2.7.3.1 PCR-based protocols

A number of PCR-based protocols have been developed for the detection of mycobacteria belonging to the Mycobacteria Tuberculosis Complex (Une and Mori, 2007). These protocols include: AccuProbe (GEN-Probe, San Diego, California, USA), the test based on DNA probe technology (Palomino *et al.*, 2007). DNA probes are species-specific, single stranded DNA oligonucleotides that are labeled with acridinum ester (chemiluminescent) that are complementary to the target of the ribosomal RNA released from bacterial cultures (Richter *et al.*, 2003). Hybridization is measured by chemiluminescence using a luminometer and expressed as relative light units (RLU). This test can be performed on culture growing from liquid or solid media and will detect all members of Mycobacterium Tuberculosis Complex (MTBC) without distinguishing among them (Somoskovi *et al.*, 2003). After DNA probe technology Line Assays were developed to expand the range of mycobacteria species identification using nucleic acid probes. The first Line Probe assay to be availed commercially was INNOLiPa Mycobacteria (Innogenetics, Ghent, Belgium). The test uses reverse hybridization technology where probes are immobilized as parallel lines on a membrane strips as opposed to being in a solution as the case with Accuprobes. In this test

amplified, biotinylated DNA fragments of the 16S-23SrRNA spacer region of Mycobacterial organisms are incubated with the labeled strips. Streptavidin-alkaline phosphatase and a chromogenic substrate are added for colouring after formation of a precipitate on the membrane where hybridization takes place (Tortoli *et al*., 1999). LiPa assay is able to detect up to 14 species of Mycobacteria (Tortoli et al., 1999). The disadvantage of LiPa assay is that it does not differentiate between mycobacteria in MTB Complex group.

Genotype *Mycobacterium* (Hain, Nehren, Germany) was developed to differentiate among mycobacteria in *Mycobaterium Tuberculosis* Complex. It is commercially availed in three different kit formats: GenoType *Mycobacterium* CM (Common Mycobacteria) to detect most frequently encountered Mycobacteria species ((Richter *et al*., 2006). Genotype *Mycobacterium* AS (Additional Species) was designed to detect less frequently encountered Mycobacteria species (Richter *et al*., 2006). GenoType MTBC DNA strip assay is able to differentiate members of MTB Complex by identifying *Mycobacterium bovis* BCG. This test uses reverse hybridization technology on a solid membrane matrix. The DNA probes are immobilized on the membrane targets polymorphisms in the gyrB DNA sequence of the Mycobacterium Tuberculosis Complex and the RD1 deletion of *Mycobacterium bovis* BCG (Richter *et al*., 2003).

2.8 Economic impact of Mycobacteriosis

Mycobacteriosis is a disease of high economic relevance within the context of livestock farming as it directly affects animal productivity as well as influencing international trade of animals and their products (Cosivi *et al*., 1998; Michel *et al*., 2010; Renwick *et al*., 2007). Further mycobacteriosis has been shown to have serious health threat for livestock, wildlife and humans (Wedlock *et al*., 2002). The disease also poses economical and financial burden to society due to economic losses through: loss of productivity of infected animals (e.g. reduced milk yields and meat production, reduced fertility), animal market restrictions,

control and eradication programs, human health costs, loss from tourism sector etc. In industrialized countries control programs have been practiced for a long time, such as meat inspection, test and slaughter of positive animals, pasteurization of milk (European Food Safety Authority (EFSA), 2004;Reviriego Gordejo and Vermeersch, 2006) thus lowering greatly economic losses due to mycobacteriosis.

Even in some industrialized countries, where mycobacteriosis has been eradicated through expensive control schemes and compensation for farmers; the disease still has a major economic impact, mainly due to the existence of a permanent wildlife reservoir that reduces the efficiency of control strategies (Thoen *et al*., 2009). Studies done in Germany estimated decrease in milk from infected cattle by 10% and in meat by 4% (Zinsstag *et al*., 2006). Studies in Bangladesh associated with a 18% decrease in milk production due to bovine tuberculosis and 4% in USA (Rahman and Samad, 2008). Similar studies were done in Canada, USA and Spain and reported loss in milk productivity (Zinsstag *et al*., 2006; Gilsdorf *et al*., 2006). In Canada losses due to reduced calves among infected cattle was reported at 15%. Gilsdorf *et al*.(2006) reported a 20% calf weight reduction. In Argentina for example, the annual loss due to mycobacteriosis is estimated to be approximately 60 million US dollars (Cosivi *et al*., 1998).

In most developing countries, where minimal or no control programs are implemented mycobacteriosis has been shown to cause severe economic losses especially in dairy cattle where there are reported mortalities, low productivity, calf mortalities, carcass condemnations and trade restrictions (Amanfu, 2006). In some countries, mycobacteriosis may affect wildlife which may in return affect animal ecosystems and tourism sector which is a major economic earner in these countries affected (Zinsstag *et al*., 2006). Public health impact has also been reported because of the zoonotic nature of the mycobacteriosis (Wedlock *et al*., 2002).

Economic losses due to mycobacteriosis worldwide is estimated at $3bln annually (Thoen and Steele, 1995). In Africa, the economic losses associated with livestock infected with mycobacteriosis have not been examined sufficiently or have not been studied at all. WHO estimated the total TB control costs in Ethiopia at US$14.2 million per year ("WHO | Global tuberculosis report 2016," n.d.).

This study will investigate the losses due to mycobacteriosis infection in slaughter house conditions mainly due to condemnation of tissues/organs/ or whole carcasses in the field setting and estimation of losses due to failed exports of animals or their products due to bTB markets restrictions. Economically the losses incurred due to mycobacteriosis will be estimated and recorded. Due to such losses, cattle owners are likely to be affected socially by reduction of their livelihoods thus applying various coping mechanisms.

3.0 MATERIALS AND METHODS

3.1 Study area

Laikipia County is located in central Kenya near the slopes of Mount Kenya. It borders Samburu County to the North, Isiolo County to the North East, Meru County to the East, Nyeri County to the South East, Nyandarua County to the south, Nakuru County to the South West and Baringo County to the West. This county lies between latitudes 0^o18" South and 0^o51" North and between longitude 36^o11" and 37^o24" East covering an area of 9,462 km.^{2} The altitude of this County varies between 1,500 m above sea level at Ewaso Nyiro basin in the North to a maximum of 2,611 m above sea level around Marmanet forest.

The county consists mainly of a plateau bordered by the Great Rift Valley to the West, the Aberdares to the South and Mt. Kenya massifs to the South East all of which have significant effects on the climatic conditions of the county. The limiting factors to agricultural and animal production are the poor weather conditions characterized by frequent dry spells and poor rainfall distribution.

The County falls mainly under agro-ecological zones LH5 ('Highland Ranching zone'), UM5 ('Livestock-Sorghum zone') and UM6 ('Midland Ranching zone'). Rainfall varies between 1200 mm (in pockets in Laikipia West) to 400mm in northern Laikipia. Life depends on two main water catchments, Aberdares range and Mount Kenya.

In land usage, 37% of Laikipia is under large scale ranching, 32% under pastoralist grazing use, 21% is under small holder farming mostly rain fed, while 0.1% is under large-scale intensive horticulture (flower & vegetable) farms.

Laikipia County comprises of five administrative sub counties namely Laikipia East, Laikipia North, Nyahururu, Laikipia Central and Laikipia West according to the present administrative

boundaries, but the map available is for the three old districts (Figure 2). The county is further sub-divided into 15 divisions, 51 locations and 96 sub-locations. According to 2009 census Laikipia County has a human population of 399,227. This population was projected to be 427, 173 persons in 2012. It is also expected to rise to 457,514 and 479,072 in 2015 and 2017 respectively. The livestock populations are as in Table 1.

Laikipia County is predominantly a pasture land with over 43 ranches occupying over 50% of the county land. Thirty ranches are either individually or company owned, while 13 are owned by the community as group ranches. Beef cattle reared are mainly Boran and to a smaller extent Sahiwal. The government had earmarked the Laikipia-Isiolo complex for the establishment of a disease-free zone as a Vision 2030 flagship project, but due to the cost and complexity of implementing it, the concept was abandoned in 2009 in favour of establishing compartments within the ranches. In the past, the county has exported live animals, semen and embryos from these ranches to Tanzania, Uganda, Zambia, Zimbabwe, South Africa and Australia. These genetics have also been sent onwards to Namibia and several countries in South America. The United States of America is also said to have imported Borans for research.

Apart from ranches the County also practices pastoralism. Two types of pastoralism are recognised: resident or agro-pastoralists, in which animals are grazed within village perimeters without migration in search of pasture, while transhumance is the most common grazing system in the region, which involves the seasonal movements of livestock to follow suitable grazing and water resources over considerable distances in the dry season, coming back to the villages in the rainy season. In the dry season, some herds remain sedentary, whereas transhumant herds often migrate together, sharing grazing areas and watering sources along the way.

The challenges affecting beef rearing includes; animal diseases such as Foot-and- Mouth disease, Contagious Bovine Pleuropneumonia, Lumphy Skin disease, and for small ruminants, PPR. Other management challenges are; drought, overstocking leading to land degradation, high cost of disease control (vaccination, tick and pest control, cost of drugs) and high cost of production especially in dry seasons where farmers have to buy pasture and feed supplements. The land invasions experienced in Laikipia County could slow down Kenya's efforts to reclaim its beef export quota to the European Union. Eight of the ranches now grappling with land invasion and uncontrolled grazing are breeding centres for the improved Boran cattle and had been declared disease-free locally. There are also fears that the invasions could affect the resumption of improved Boran cattle embryo exports to South Africa and lead to loss of genetics developed over many years.

Table 1: Livestock Population in Laikipia County, Kenya

District	Cattle	Sheep	Goats	Camels
Laikipia North	39,417	86,452	120,416	2,064
Laikipia East	39,500	58,750	38,000	200
Laikipia Central	55,550	79,800	59,900	800
Total	134,467	225,002	218,316	3,064

Figure 3: Map of Laikipia County

3.2 Study design

This study was carried out in Laikipia County between January and July, 2015 to estimate the prevalence of bovine mycotuberculosis, it's etiology, public health importance and economic impact. The study covered three out of the four sub-counties (North, East and Central). Nanyuki municipal abattoir was used for meat inspection and lesion sampling from the slaughtered bovine carcasses. This abattoir was selected being the only main slaughter house serving the three sub-counties. It was anticipated that this was the most likely place where

cattle for slaughter are received from the whole county, and this would largely reflect the mycobacteria strains circulating in the county.

3.2.1 Sample Size Determination

To generate a representative sample size, this study assumed the average prevalence of bovine tuberculosis at 2% as documented by Gathogo *et al.*, (2012). The study assumed a 95% confidence interval (Thrusfield, 2005). From a total of 7,564 cattle carcasses slaughtered, 1000 were sampled using simple random sampling method, as by the formula below:

$$n = Z^2 \ P(1 - P)/d^2$$

n = required sample size

Z = value for the corresponding confidence level (e.g., 1.96 for 95% confidence)

P = estimated value for the proportion of a sample/expected prevalence Gathogo *et al.*(2012)

d= is the margin of error

d=0.0l [d=l/2P where P<0.1, (Thrusfield, 2005), quoted by Gathogo *et al.*(2012)

 P= 2% (0.02)   where:

n=1.96^2 * 0.02(1-0.02)/0.01^2

n= **753 animals** (However the number of animals sampled was 1000).

3.3 Sampling procedure

Post mortem meat inspection was carried out according to procedures established by legislation (Meat Control Act, cap.356). Inspection of each carcass was undertaken in detail. Particular emphasis was given to the head and thoracic lymph nodes, lungs, pleura, peritoneum, abdominal organs and mesenterium. In the head, parotid, sub-maxillary, and retropharyngeal lymph nodes were palpated, incised and examined.

In the thoracic cavity, the pleura was examined, the apical, bronchial anterior and posterior lymph nodes were palpated, incised and examined. The lung tissue was also palpated, incised

and examined. In the abdominal cavity, the spleen, liver and portal lymph nodes were palpated, incised and examined. Kidneys were palpated, de-capsulated, examined and if necessary incised.

Mesenteric lymph nodes were randomly palpated, incised and examined. In the carcasses; prescapular (superficial cervical), superficial inguinal in males and supra-mammary in females, iliac lymph nodes were palpated, incised and examined.

The incised surfaces were examined under bright light for the presence of yellowish appearance, abscess, cheesy mass and calcification. Localised tuberculous lesions in the various parts of affected organ(s) or carcasses led to partial condemnation of the affected part and if generalised, led to total condemnation.

Lesions suggestive of mycobacteriosis (granulomatous, yellowish, purulent, caseous, or calcified in consistency) were excised, trimmed and packed into sterile 50ml Falcon^R tubes, labelled, placed into a cooler-box with ice, transported to the laboratory and preserved at -20^0C till processing. Large samples were cut into approximate cube of one centimetre. In this study no ethical clearance was required because condemnation of diseased organs and carcases was part of a routine meat inspection procedure.

3.4 Processing of the samples in the laboratory

Before processing, tissue samples were allowed to thaw, by removing them from the freezer to the fridge temperature (+4 ^{0} C) a day before processing. The samples were processed at the National Tuberculosis Reference Laboratory (NTRL), Ministry of Health, Nairobi, according to the OIE Standard Procedures (OIE, 2009) in a Bio-Safety Cabinet (BSC) level II.

The specimens were homogenized in sterile Griffith tissue grinder tubes. Approximately 5ml distilled water was added, sample was homogenized, and 5ml aliquots of the homogenates were transferred into 50ml Falcon^R tubes and equal volume of 4% Sodium hydroxide

(NaOH) added to decontaminate other bacteria. The tubes were then tightly closed, vortexed for 5 minutes and left to stand for another 10 minutes to allow aerosols to settle.

After the 15 minutes of decontamination, phosphate buffered saline (PBS), pH 6.8 was added to the 45 ml mark of the Falcon^R tube to stop decontamination. Then suspension was centrifuged at 800 xg for 30 minutes at 4 ^{0}C. The supernatant was discarded into 5% phenol solution and the pellet completely re-suspended in 3ml PBS containing benzylpenicillin, 50 IU per ml, by vortexing to complete dissolution of the pellet.

3.4.1 Direct Ziehl-Neelsen (ZN) Staining

The suspensions were stained using Ziehl-Neelsen (ZN) staining method following standard procedure and examined for acid fast bacilli (AFB). Briefly, a loop full of the suspension was placed on a labelled clean grease free slide, thinly spread on the slide and left to air dry. ZN staining was then carried out following the standard procedure. Stained slides were examined under oil immersion.

3.4.2 Culturing

Approximately 200µl of the suspension were inoculated into three tubes of Loweinstein-Jenseen (L-J) slant media containing either 0.4% sodium pyruvate, 0.75% glycerol, or para-nitro benzoic acid (PNB) at 0.5 mg/ml. The media slats were incubated at 37^{0}C for up to 8-12 weeks with weekly observation for growth after four weeks. Colonies were then examined for AFB.

3.4.3 Molecular analysis

GenoType^R *Mycobacterium* DNA strip assays were used for molecular analysis, to isolate DNA from positive cultures, following HAIN Lifescience manual (HAIN Diagnostica, Nehren, Germany). Three kits were used separately, the GenoType *Mycobacterium* CM (Common Mycobacteria), GenoType *Mycobacterium* AS (Additional Species) allowing

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identification of 17 species of Mycobacteria Other Than Tuberculosis (MOTTs) and the GenoType *Mycobacterium* MTBC (Mycobacteria Tuberculosis Complex species) that identifies mycobacterial species in the MTBC group (Bouakaze *et al*., 2010).

3.4.3.1 Isolation and characterization of Mycobacteria Other Than Tuberculosis (MOTTs) using GenoType *Mycobacterium* CM

DNA extraction
About three colonies of bacteria was collected from each AFB-positive culture and suspended in 300μl of molecular grade water in sterile labelled 1.5ml microcentrifuge tubes and then tightly closed. The manipulations were carried out in a BSC cabinet. The tubes were fixed in a rack and incubated for 20 minutes in boiling water (95^0 C), followed by incubation in an ultrasonic bath for 15 minutes at room temperature. This procedure was meant to inactivate and to break the mycobacterial cell wall to release DNA. The tubes were then centrifuged at 11,000 xg for 5 minutes at room temperature and the supernantant, containing the template DNA was then transferred to a fresh cryovial tube and stored at +4 ^{0}C till use. DNA analysis was done in three levels, using GenoType^R *Mycobacterium* DNA strip assay kits (HAIN Diagnostica, Nehren, Germany). Samples were first analyzed using GenoType ^{R} *Mycobacterium* (CM), followed by GenoType^R *Mycobacterium* AS kit and species identified by the two kits were then speciated using GenoType^R *Mycobacterium* MTBC (*M. tuberculosis* complex) kit.

DNA amplification
Master mix preparation was done in a DNA cabinet. Thirty five microlitres (35μl) Primer nucleotide mix (AM-B) and 10 μl of AM-A mixture were accurately measured into a sterile 1.5 μl Eppendorf tube to make aliquots of the master mix. Aliquots (45μl) were then transferred to a biosafety cabinet class II. For CM and AS analysis, 100 μl of each DNA sample was first mixed with 2 μl of internal control DNA in a different labelled PCR tubes. For MTBC analysis, 5 μl neat DNA template samples were transferred directly into PCR

tubes. The tubes were then loaded into a thermocycler (Techne TC-5000, Bibby Scientific Ltd.,China). The thermocycler line probe programme was selected for samples of the culture origin ("MDR CUL"). The thermocycler was lid heated at $105^{o}C$ for 3mins before amplification. Amplification protocol for CM and AS analysis consisted of one denaturation cycle of 15 minutes at $95^{o}C$, followed by 10 cycles comprising 30 s at $95°C$ and 2 min at $65°C$, an additional 20 cycles comprising 25 s at $95°C$, 40 s at $50°C$, and 40 s at $70°C$, and a final extension of 8 min at $70°C$.

Amplification profile is summarised as follows:

15min	$95^{o}C$	1 cycle
30sec	$95^{o}C$	10cycles
2min	$65^{o}C$	
25sec	$95^{o}C$	
40sec	$50^{o}C$	20cycles
40sec	$70^{o}C$	
8min	$70^{o}C$	1 cycle

The program ran for 2.0 hours. The PCR tubes were then removed, placed on a rack and transferred to hybridization area.

Hybridization and evaluation
Hybridization and detection procedure were uniform for all three assays and were carried out using semi-automated washing and shaking device (Twincubator[R], Hain Lifescience, Nehren, Germany). Preparation before hybridization included pre-warming of the hybridization buffer (HYB), strigent wash solution (STR) and TwinCubator to $45^{o}C$. Aliquotes of 20 µl aliquot of denaturation solution (DEN) was dispensed into one corner of the wells in a plastic reaction tray. Twenty (20) µl of amplified product (amplicon), from each corresponding sample was then added to the DEN solution in each tray and gently mixed. The mixture was incubated at room temperature for 5 minutes. One ml of pre-warmed hybridization buffer (HYB) was added into each well and allowed to mix with the DEN-amplicon mixture. Strips were

removed from their respective kits, labelled with a fine pencil and then loaded into its corresponding well by use of forceps. Each numbered test strip was fully submerged into the mixture. The loaded reaction tray was then incubated for 30 minutes at 45^0 C in a Twincubator^R. HYB buffer was then removed by inverting the tray above a sink and then completely aspirated. One ml of stringent wash solution (STR) was added into each well, placed on the TwinCubator and incubated for 15 minutes at 45^0 C. STR was then removed by inverting the tray above a sink and placing it on absorbent paper towel.

Each well was then rinsed with 1ml of rinsing solution (RIN) by incubating for 1 minute and then discarding by inverting the reaction tray. 1ml of diluted conjugate (CON) was added into each well and incubated at 45^0 C for 30 minutes in the TwinCubator. CON solution was discarded by inverting the reaction tray followed by two successive rinsing using RIN solution each for 1 minute and twice rinsed using distilled water for one minute. 1ml of diluted substrate was then added into each well and incubated in a dark place for 3-20 minutes and then discarded by inverting the reaction tray and washing with distilled water. Hybridized products were developed by addition of a conjugate buffer which contains streptavidin conjugated with alkaline phosphatase and a subsequent addition of the substrate buffer for colorimetric detection of the bands. After a final washing, strips were air dried and fixed on blot paper. Each strip contains 17 reaction zones as displayed below (figure 3). The developed strips were pasted in the designated fields by aligning the CC, IC and GC bands with the respective lines on the evaluation sheet provided by the manufacturer.

Figure 4: Strip banding pattern for Mycobacteria Other Than Tuberculous (MOTTs) using GenoType *Mycobacterium* CM

Conjugate Control (CC)- presence of this line shows the efficiency of conjugate binding and substrate reaction

Internal Control(IC) –presence of this line validates the test

Genus Control (GC) – Staining of this zone shows the presence of *Mycobacterium* genus

Other reaction zones – Are for specific probes

3.4.3.2 Isolation and characterization of Mycobacteria Other Than Tuberculosis (MOTTs) using GenoType *Mycobacterium* AS

DNA extraction
DNA extraction was carried out as for GenoType *Mycobacterium* CM.

DNA amplification
Amplification was carried out as for GenoType *Mycobacterium* CM

Hybridization and evaluation
Hybridization was carried out as described for GenoType CM.
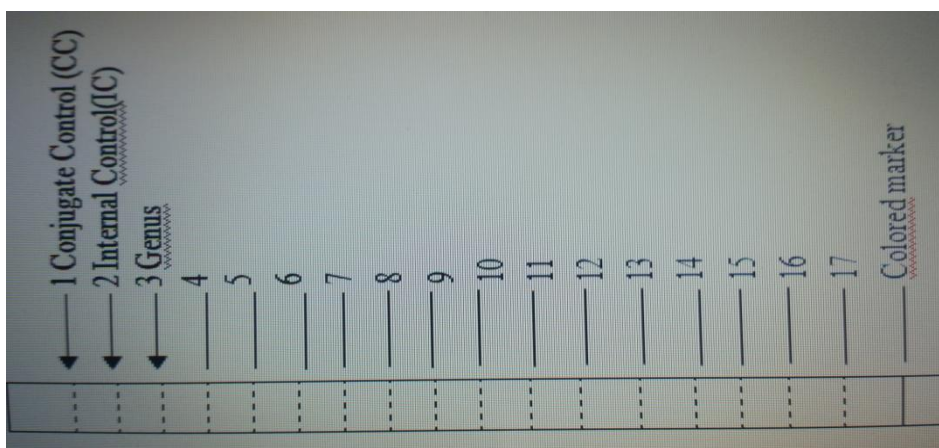
Figure 4: Strip banding pattern for Mycobacteria Other Than Tuberculous (MOTTs) using GenoType *Mycobacterium* AS

Conjugate Control (CC)- presence of this line shows the efficiency of conjugate binding and substrate reaction

Internal Control(IC) –presence of this line validates the test

Genus Control (GC) – Staining of this zone shows the presence of *Mycobacterium* genus

Other reaction zones – Are for specific probes

Each strip contains 17 reaction zones as displayed below (figure 4). The developed strips were pasted in the designated fields by aligning the CC, IC and GC bands with the respective lines on the evaluation sheet provided by the manufacturer.

3.4.3.3 Characterization of Mycobacteria using GenoType MTBC

DNA extraction using GenoType MTBC kit
DNA extraction was carried out as for GenoType *Mycobacterium* CM/AS.

DNA amplification
Mastermix preparation (45 µl) for each sample was carried out as described for GenoType CM. DNA was carefully transferred to the PCR tubes containing mastermix and transferred to the amplification room.

The thermocycler (Techne TC-5000, Bibby Scientific Ltd.,China) programme was selected (Genotype MTBC Hain). The thermocycler was lid heated at 105°C for 3mins before amplification. Amplification protocol for MTBC consisted of one cycle 15 min. of denaturation at 95°C, followed by 10 cycles comprising 30 s at 95°C and 2 min at 58°C, an

additional 20 cycles comprising 25 s at 95°C, 40 s at 53°C, and 40 s at 70°C, and a final

extension of 8 min at 70°C. The program ran for 2.5 hours. The PCR tubes were removed,

placed on a rack and transferred to hybridization area.

Amplification profile is summarised as follows:

15min	95°C	1 cycle
30sec	95°C	10cycles
2min	58°C	
25sec	95°C	
40sec	53°C	20cycles
40sec	70°C	
8min	70°C	1 cycle

Hybridization and evaluation
Hybridization was carried out as described for GenoType CM/AS. Each evaluation strip

contains 13 reaction zones as displayed below (figure 5). The developed strips were pasted in

the designated fields by aligning the CC and UC bands with the respective lines on the
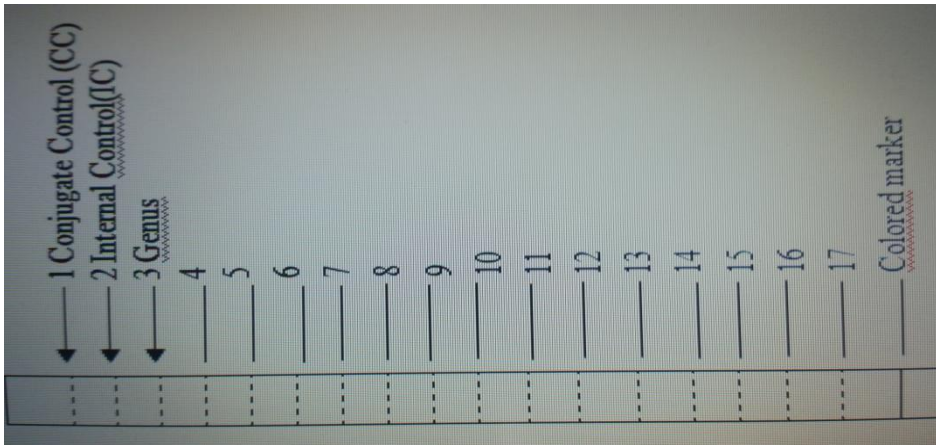
evaluation sheet provided by the manufacturer.
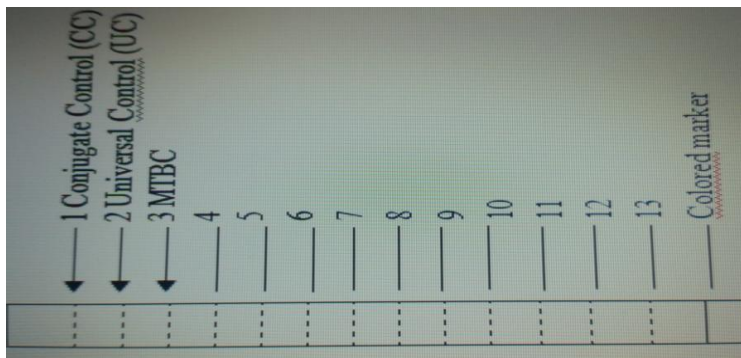
Figure 5: Strip banding pattern for Mycobacteria Other Than Tuberculous (MOTTs) using
GenoType MTBC

Conjugate Control (CC)- presence of this line shows the efficiency of conjugate binding and
substrate reaction:

Universal Control (UC) – shows the presence of Mycobacteria and members of gram-Positive
bacteria with a high G+C content

MTBC – Staining of this zone shows the presence of *Mycobacterium tuberculosis* complex

Other reaction zones – Are for specific probes

3.5 Determination of economic impact by estimating the loss through condemnation of carcasses/organs and lost trade opportunities.

The economic impact was determined by estimating losses through condemnation of organs/carcasses at slaughter houses and lost trade opportunities due to threat of BTB at National level. During meat inspection exercise at the slaughter house(s), every organ/tissue/carcass condemned due to suspected bTB was weighed, recorded and the monetary value of it determined by multiplying the approximate weight with the market value of meat. This was done on daily bases and total sum was recorded and calculated at the end of the study period. A desktop study was conducted at Director of Veterinary Services (DVS) office to collect and collate the information on losses of export opportunities due to bovine tuberculosis threat in Kenya. Trade inquiries featured the number of live livestock or meat trade for the last ten (10) years. The number of inquiries that succeeded and failed were recorded. Those that failed due to lack of tuberculosis surveillance and control programmes in cattle were recorded and converted into economic/monetary values.

3.6 Data analysis

Data on lesion location, consistency, size, and weighed condemned tissue/organ were recorded on a data sheet during post mortem examination. The data was entered into an MS-Excel, cleaned, coded, and analyzed using SPSS package 23. The prevalence of the disease was calculated for tuberculous lesion, ZN staining, culture, organs/tissues condemned and molecular analysis. Prevalence was determined as a proportion (Thrusfield, 2005). Sensitivity and specificity of the different diagnostic methods in relation to mycobacteria growth was calculated and expressed in percentages. Statistical analysis included comparison of prevalence proportions of different methods done. Chi-square (χ^2) test was used to test relationship between mycobacteria growth against lesion size, lesion consistency and direct

ZN staining for tuberculous lesions. Kappa(φ) test was used to test agreement between ZN staining and bacteriological recovery in different culture media. For all the analysis performed the confidence level was 95% and precision (P-value) of 5% or less was considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Tuberculous lesions in meat carcasses

A total of 1000 bovine meat carcasses were sampled and examined for tuberculous lesions. Two hundred and eighteen (21.8%) [95% CI: 19.35-24.46] of the carcasses sampled were found to have lesion located in one or more organs/tissues. In the affected organs or tissues the lesions were either single or multiple. Figure 6 shows multiple tuberculous lesions in the lungs.
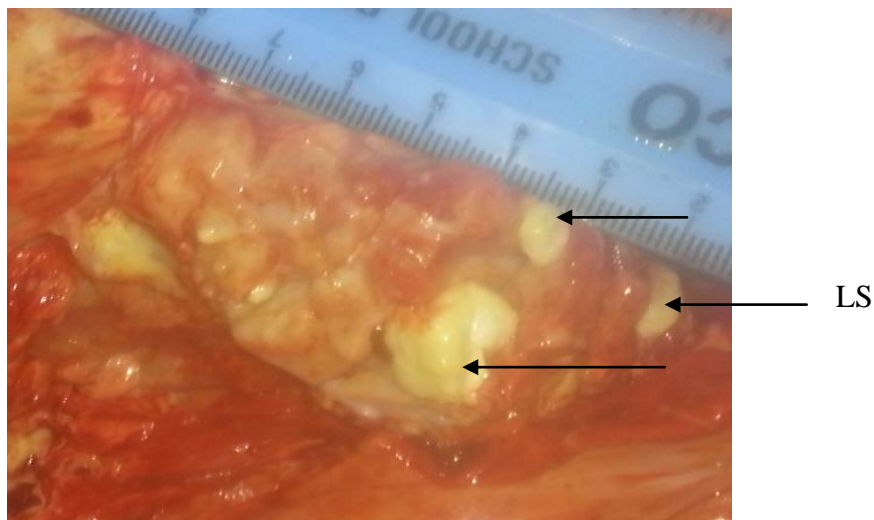
Figure 5: Multiple caseous lesions (Ls) in the lungs from a cow meat carcass, Laikipia County, Kenya.

Lesions were observed in the lymph nodes of the head region, specifically, retropharyngeal, parotid and submandibular 13 (6%), bronchial lymph nodes 13 (6%), mediastinal lymph nodes 16 (7.3%), lungs 97 (44.5%), liver and portal lymph nodes 49 (22.5%) and mesenteric lymph nodes 2 (0.9%). Other organs affected were spleen and aortic serosa 28 (12.8%).

The size of the lesions ranged from small (0–5mm), medium (5-10mm) or large (>10mm), and the consistency was either caseous (17.9%), fibro-caseous (27.1%), fibro-calcified

(8.3%) or calcified (46.8%). A summary of lesion location and consistency is summarized in

table 2.

Table 2: The location and consistency of tuberculous lesions in cattle meat carcasses
examined in Nanyuki Munincipal abattoir Laikipia, County.

Lesion location	Number (percentage)	Consistency				Total
		Caseous	Fibro-caseous	Fibro-calcified	Calcified	
Head Lns	13 (6%)	2	4	0	7	13
Bronchial Lns	13 (6%)	1	2	2	8	13
Mediastinal Lns	16 (7.3%)	4	7	1	4	16
Lung	97 (44.5%)	17	23	8	49	97
Liver and portal Lns	49 (22.5%)	12	15	5	17	49
Mesenteric Lns	2 (0.9%)	0	1	0	1	2
Others	28 (12.8%)	3	7	2	16	28
Total	218 (100%)	39 (17.9%)	59 (27.1%)	18 (8.3%)	102 (46.8%)	218 (100%)

Key: Lns=lymph nodes

4.2 ZN staining

On examination of direct smears stained with ZN staining method, acid fast bacilli (AFB)

were observed in 63 of the 218 lesion smears. The proportion on the basis of ZN staining was

therefore 63/1000 (6.3%) [95% CI:4.95-7.98%] (table 4). The AFBs in the smears ranged

from scanty to numerous (Figure 7).
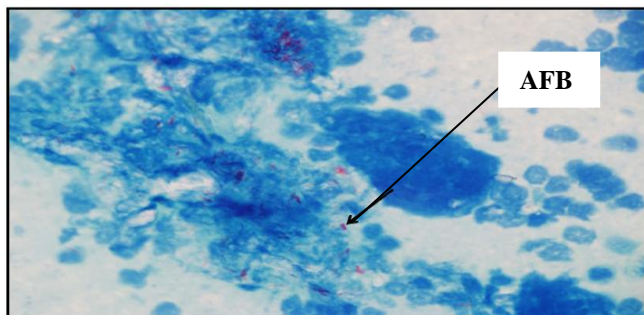
Figure 6: ZN stained direct smear of a tuberculous lesion in a mediastinal lymph node from a
cow meat carcass showing presence of acid fast bacilli (AFB).

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A Chi-square test (χ^2) analysis of the relationship between the ZN staining and lesion consistency ($\chi^2 = 8.450$; p=0.197); while ZN staining and lesion size ($\chi^2 = 0.820$; p=0.061) indicated no statistical significance as summarized in Table 3.

Table 3: Consistency, size and direct ZN staining of tuberculous lesions from cattle meat carcasses examined in Nanyuki Municipal abattoir, Laikipia County.

Lesion consistency /Size	Small (0-5mm)		Medium (5-10mm)		Large(>10mm)		Total	
	ZN+ve	ZN-ve	ZN+ve	ZN-ve	ZN+ve	ZN-ve	ZN+ ve	ZN-ve
Caseous	2	1	3	14	3	16	8	31
fibro-Caseous	2	6	3	6	14	28	19	40
Fibro-calcified	2	1	2	2	6	5	10	8
Calcified	6	14	11	29	9	33	26	76
Total	12 (5.5%)	22 (10.1%)	19 (8.72%)	51 (17%)	22 (23.4%)	82 (37.6%)	63 (28.9%)	155 (71.1%)

Key: ZN +ve =Ziehl-Neelsen positive, ZN –ve = Ziehl-Neelsen negative

4.3 Mycobacteria culture

Isolates were recovered from 50/1000 (5%) [95% CI:3.7-6.5] of the lesion samples. Of these isolates, 30/50 were recovered in pyruvate media, 19/50 in para-nitro benzoic acid (PNB) and 10/50 in glycerol media. Seven isolates were recovered in both pyruvate and PNB and two on both glycerol and pyruvate, while one was recovered in all three media. Figure 8 shows mycobacteria isolate colonies on pyruvate media.
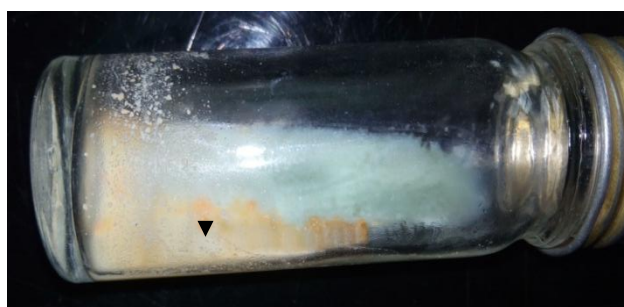
Figure 7: *Mycobacterium* colonies on pyruvate L-J solid media, isolated from a tuberculous lesion in a cow meat carcass.

Among all lesions that yielded acid fast bacilli only 3/63 (4.8%) had growth on glycerol, on pyruvate 10/63 (15.9%) and 10/63 (15.9%) on PNB as shown in table 5. The sensitivity and specificity on isolation and identification of the mycobacteria was 20.6% and 76.1% respectively with kappa value of zero (K=0). The kappa value (k=0) shows no agreement at all between acid fast staining results (ZN) and bacteriological recovery. Chi-square test (χ^2) analysis of the association between the mycobacteria growth and lesion consistency ($\chi^2 = 0.789$); and lesion size ($\chi^2 0.095$) while mycobacteria growth and ZN staining ($\chi^2 = 0.0606$) indicated no statistical significance.

Table 4: The comparison between direct ZN staining and mycobacteria growth on three types of L-J media of tuberculous lesions from cattle meat carcasses.

ZN Staining	Glycerol			Pyruvate			PNB		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
Positive	3	60	63	10	53	63	5	58	63
Negative	7	148	155	20	155	175	14	141	155
Total	10	208	218	30	208	218	19	199	218

Caseous lesions recorded nil mycobacteria growth; while fibro-caseous had 9, fibro-calcified 2 and calcified 12 as summarized in table 5.

▼

Table 5: The consistency, direct ZN staining and mycobacteria growth on three types of L-J media of tuberculous lesions from cattle meat carcasses.

Consistency of the lesion	Mycobacterium recovery							
	Growth on glycerol		Growth on pyruvate		Growth on PNB		Total	
	ZN +ve	ZN-ve	ZN +ve	ZN -ve	ZN +ve	ZN -ve	ZN +ve	ZN -ve
Caseous	0	2	0	8	0	8	0	18
Caseo-fibrous	1	2	4	3	4	3	9	8
Fibro-calcified	0	0	1	0	1	0	2	0
Calcified	2	3	5	9	5	9	12	21
Total	3	7	10	20	10	20	23	47

Key: ZN +ve =Ziehl-Neelsen positive, ZN −ve = Ziehl-Neelsen negative

4.4 DNA analysis

DNA analysis identified Mycobacteria in 35/50 (70%) of the isolates. PCR GenoType Mycobacterium (CM) kit identified *Mycobacterium fortuitum*, *Mycobacterium interjectum*. AS kit identified *Mycobacterium shimoidei*, *Mycobacterium celatum*, *Mycobacterium szulgai*, *Mycobacterium asciaticum*, *Mycobacterium* kansaasii and Mycobacterium *species*. GenoType® MTBC identified *Mycobacterium bovis*. The most frequent mycobacteria was *Mycobacterium fortuitum* 12/50 (24%), followed by *Mycobacterium bovis* 3/50 (6%), *Mycobacterium shimoidei* 2/50 (4%), while *Mycobacterium interjectum*, *Mycobacterium asciaticum, Mycobacterium celatum, Mycobacterium szulgai* and *Mycobacterium kansaasii* were 1/50 (2%) each. Thirteen (13) of Mycobacterium isolates could not be speciated. The prevalence of zoonotic mycobacteriosis was therefore 0.3% (3/1000) [95% CI: 0.1-0.88] and overall MOTTs Mycobacteriosis was 3.2% (32/1000) [95% CI: 2.28-4.48]. Among MOTTs; *Mycobacterium fortuitum* was 1.2% (12/1000), *Mycobacterium species* 1.3% (13/1000), *Mycobacterium shimoidei* 0.2% (2/1000), and *Mycobacterium interjectum, Mycobacterium*

asciaticum, Mycobacterium celatum, Mycobacterium szulgai, Mycobacterium kansaasii was 0.1% each (1/1000). The overall prevalence of Mycobacteriosis in this study was 3.5% (35/1000) [95% CI: 2.53-4.83].

Table 6 shows the results of the DNA analysis of the isolates. Figure 9a, b and c shows strip banding patterns of the various isolates.

Table 6: DNA analysis of mycobacteria isolated from tuberculous lesions from cattle meat carcasses examined in Nanyuki Munincipal abattoir, Laikipia County.

Lesion location	*M. fortuitum*	*M. bovis*	*M. shimoidei*	*M. interjectum*	*M. asciaticum*	*M. szulgai*	*M. celatum*	*M. kansaasii*	*M. species*	Negative	Total
Head Lns	1	1	0	0	0	0	0	0	0	0	2
Bronchial Lns	0	0	0	0	0	0	0	0	2	1	3
Mediastin-al Lns	2	0	0	0	0	0	0	0	1	1	4
Lungs	3	1	1	0	1	1	1	0	3	10	21
Livers and portal Lns	2	1	1	1	0	0	0	0	6	1	12
Mesenteric Lns	0	0	0	0	0	0	0	0	0	0	0
Other organs	4	0	0	0	0	1	0	1	1	2	8
Total	12	3	2	1	1	1	1	1	13	15	50

Figure 9 a, b and c shows strip banding patterns of the various isolates

Figure 9 a: GenoType® *Mycobacterium* CM analysis results for mycobacteria isolated from cattle meat carcasses. Note the banding patterns for *M.fortuitum* **1,2,3,7,14,** *M.interjectum* **1,2,3,9,10,11** and positive control (*M.kansaasii*) **1,3,9,10,12**
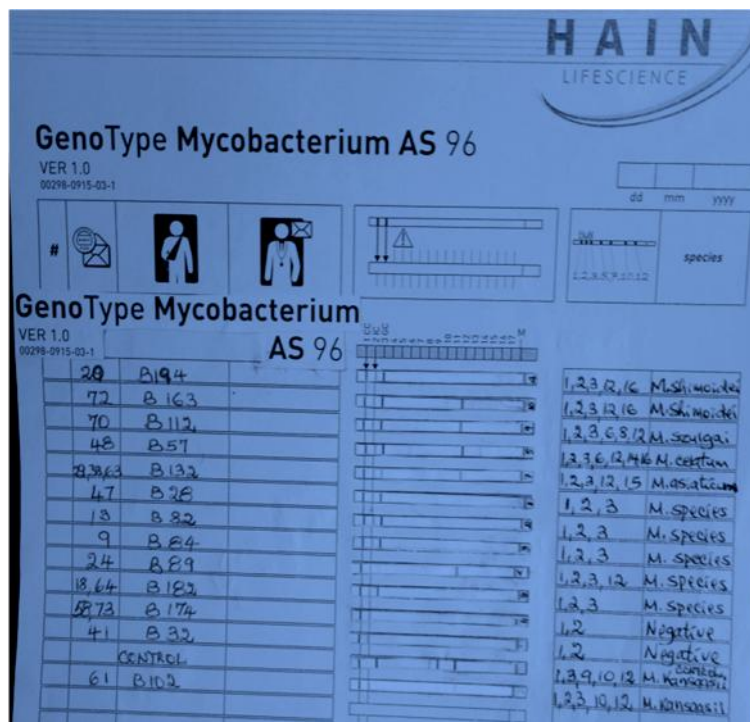
Figure 9 b: GenoType® *Mycobacterium* AS analysis results for mycobacteria isolated from cattle meat carcasses. Note banding patterns for *M. Shimoidei* **1, 2, 3, 12, 16** ; *M. Szulgai* **1,2,3,6,8,12** ; *M. Celatum* **1,2,3,6,12,14,16** ; *M.kansaasii* **1,2,3,10,12** ; *M. species* – **1,2,3,(12)**, negative **1,2** and positive control (*M.*kansaasii) **1,3,9,10,12**.
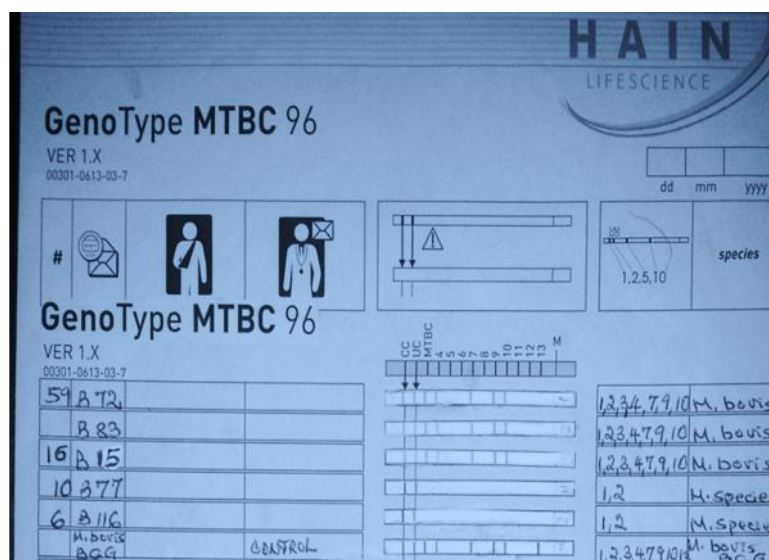
Figure 9 c: GenoType® MTBC analysis results for mycobacteria isolated from cattle meat carcasses. Note banding patterns for *M. bovis* **1, 2, 3, 4, 7, 9, 10**; *M. species* – **1, 2** and positive control (*M. bovis* BCG) – **1,2,3,4,7,9,10,13**

In summary, overall prevalence of tuberculous lesions was 21.8% [CI: 19.35-24.46]. On DNA analysis; prevalence was 3.5% [CI: 2.53-4.83] for mycobacteriosis, 0.3% [CI: 0.1-0.88] for zoonotic mycobacteriosis and 3.2% [CI: 2.28-4.48] for MOTTs mycobacteriosis.

4.5 Economic impact

Assessment of losses due to condemnations of organs/tissues at the slaughter house totalled 422 kilograms. Organs condemned were mainly livers (219kilograms), followed by lungs (132 kilograms), muscle tissues (71 kilograms). At the time of the study a kilogram of beef was selling at Kenya shillings 400. The estimated loss stood at Kenya shillings 168,800 KSHS for the duration of the study period. This amounted to approximately Kenya shillings 506,400 (506 USD) per annum. At the national level there were 196 enquiries for livestock export during a ten year enquiry period. Most of the trade enquiries involved animal products such as meat, pet foods of animal origin and fresh milk and milk products. Two of the failed trade involved live animals (4500 H/C and 10 camels). Monetary loss was approximated at 405 million Kenya shillings (KHS), where one animal value was approximated at 90,000

Kenya Shillings. Failure of trade with live cattle was due to lack of evidence to demonstrate zoonotic tuberculosis surveillance and control programme.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

This study involved collection of samples with tuberculous lesions from the slaughter house and laboratory sample analysis to determine the prevalence, aetiology, public health importance and a desktop study to determine the economic impact of mycobacteriosis in slaughter cattle.

5.1 Prevalence of tuberculous lesions in cattle meat carcasses

In this study, tuberculous lesions were observed in 21.8% (218/1000) [95% CI: 19.35-24.50] of the meat carcasses. These findings are comparable with those observed in Ethiopia that reported a prevalence of 23.9% (Demelash *et al*., 2009). However the work done by Demelash in Ethiopia had showed strong correlation with management systems, where those confined reported a high prevalence proportion of the tubercle like lesions. The current study didn't put in account risk factors. This study also agreed with an earlier study that was done in Kenya that reported a prevalence proportion of 18.95% (Gathogo *et al*., 2012a).

However this study found a higher prevalence than reported by Nwanta *et al*.(2011), who recorded 14.9% gross tuberculous in Nigeria; Worku *et al*.(2016) in Ethiopia (15.9%); Boukary *et al*.(2012) in Niamey, Niger (0.19%); Asil *et al*.(2013) in Sudan (0.18%); Habarugira *et al*.(2014) in Rwanda (0.9%) and Nalapa *et al*.(2017) in Mubende District, Uganda (9.7%). The current study however reported a lower prevalence than the 47% reported by Biet *et al*.(2005), in France. This lower prevalence could be due to the low number of tissues inspected and the relatively high proportion of small lesions which could not be detected in routine and detailed meat inspection (Tintagu Gizaw, 2017).

Various studies in Africa have identified various risk factors for mycobacteriosis transmission at different spatial levels. Males were more significantly more affected by bTB than female animals (Kazwala *et al*., 2001). Bos indicus (zebu) have been found to be more

resistant to bTB than Bos Taurus (Exotic breeds), where imported dairy animals are generally kept under intensive management conditions (Firdessa *et al*., 2012). Older cows were more likely to have been exposed as younger ones, as shown by several cross-sectional studies carried out in Tanzania, Zambia and Chad (Cleaveland *et al*., 2007; Cook *et al*., 1996; Müller *et al*., 2008; Inangolet *et al*., 2008). Nomadic transhumance has also been cited as a risk factor where livestock are moved together as several herds to follow grazing and water. This increases contact between animals, raising chances of infection (Tschopp *et al*., 2009). In conclusion, the current study however reported a lower prevalence (21.8%) than the 47% reported by Biet *et al*.(2005), in France.

5.2 Distribution of lesions

Lesions were observed in the lymph nodes of the head region, specifically, retropharyngeal, parotid and submandibular 13 (6%), bronchial lymph nodes 13 (6%), mediastinal lymph nodes 16 (7.3%), lungs 97 (44.5%), liver and portal lymph nodes 49 (22.5%) and mesenteric lymph nodes 2 (0.9%). Other organs affected were spleen and aortic serosa 27 (12.4%).

Tuberculosis-like lesions were predominantly in the lungs and associated lymph nodes (57.8%), which was in agreement with previous studies (Romero *et al*., 2011; Shitaye *et al*., 2006, Amen *et al*.,2011; Gathogo *et al*.,2012). Lungs and bronchial lymph nodes recorded a sum of 31.6% (69) tuberculous lesions. This agreed with other studies done elsewhere (Asseged *et al*., 2004; Awah Ndukum *et al*., 2010; Dechassa, 2014; Opara *et al*., 2012; Worku and Abreham, 2016). However, the above results disagreed with other studies done previously (Murphy *et al*., 2010) that found lymph nodes in the head to be most frequently infected.

According to Corner, (1994), up to 95% of cattle with visible tuberculosis lesions could be identified by examination of lungs and associated lymph nodes (bronchial, mediastinal, retropharyngeal). Therefore, during post mortem meat inspection examination, focus should

be given on lungs and associated lymph nodes. In addition, the lung, liver, spleen and the surfaces of body cavities are commonly affected. However, the presence of lesions in others organs indicates that the infection also occurs through ingestion of contaminated pastures, water and feeds (Tintagu Gizaw, 2017). A study done by Humble and colleagues reported that lack of hygiene predisposes to the proliferation of pathogens in general (Humble *et al*., 2009). A presumptive diagnosis of bovine mycobacteriosis can be made following the macroscopic detection of these granulomatous lesions in different organs of infected animals (Ramos *et al*., 2015).

According to the results in this study, majority of the lesions were found in the lungs and associated lymph nodes (57.8%), which may indicate higher aerosol infection route than oral (ingestion) route. Animals examined in this study were raised under nomadic transhumance production system where livestock are moved together as several herds to follow grazing and water during drought seasons. During hot weather animals gather together under shades and at night crowd for warmth and security. This increases contact between animals raising chances of infection (Tschopp *et al*., 2009).

5.3 Consistency of the lesions

Consistency of tuberculous lesions, ranged from caseous (17.9%), fibro-caseous (27.1%), fibro-calcified (8.3%) and calcified (46.8%). The granulomas types may show indication of level of immunity of animal and the duration of infection (Flynn and Klein, 2010). The pathology of tuberculosis is characterized by the formation of granulomas which can within the course of the disease regress or exhibit extensive necrosis, calcify or liquefy and subsequently lead to cavity formation (Admassu, *et al*., 2015).

Macroscopically, a tuberculous granuloma usually has a yellowish appearance and is caseous, caseo-calcareous, or calcified in consistency. Occasionally, its appearance may be more purulent in cervids and camelids (OIE, 2009). In this investigation majority of the lesions

were calcified (46.8%), probably indicating a level of immunity of animal and a long duration of the infection (Flynn and Klein, 2010).

5.4 Direct ZN staining

Direct Ziehl-Neelsen (ZN) staining confirmed 28.9% (63/218) AFB positives of all the tuberculous lesions analysed, which translates into 6.3% [95% CI: 4.9-8.0] of the 1000 cattle carcasses sampled. This value was comparable to that observed by Saidu *et al.*, (2015), Nigeria where 29.16% AFB was reported; in Algeria, where 28.85% was reported (Sahraoui *et al.*, 2009) and in Cameroon where 31% was reported (Awah Ndukum *et al.*, 2010). However these results were higher than those reported by Nuru *et al.*(2017) who observed 17.7% AFBs North Western Ethiopia. Acid fast bacilli (AFB) microscopy is cheap, requires little material and gives results within a day. On the other hand, the method is tedious, time consuming and lacks sensitivity. Individual studies have reported a great disparity in the sensitivity values, from 33% to 80% (Nour-Neamatollahi *et al.*, 2018). According to Nour-Neamatollahi *et al.* (2018), only 45.6% of all TB cases in 2004 were smear-positive. *Mycobacterium* often may be found in low numbers, due to sample taking technique during smear preparation as mycobacteria are not evenly distributed in the tissue sample, resulting to not being visualized after staining (Bekele and Belay, 2011). There are other pathogens that macroscopically produce tuberculous like lesions, such as *Actinomycetes* apart from mycobacteria such as *Actynomices bovis*, *Trueperella pyogenas*, *Corynebacterium, Gordonia, Nocardia, Rhodococcus* and *Tsukamurella, Dietzia,* fungi, parasites such as sarcocystis, hydatids (Sokolovská *et al.*, 2003; Biet *et al.*, 2005). This may explain why not all tuberculous lesions turned ZN positive. In completely calcified lesions, ZN may stain negative due to low sensitivity of acid-fast staining at detecting low numbers of organisms, or because of the presence of non-acid-fast forms of the organism, or might reflect a genuine absence in the necrotic area of the lesion (Bryan Markey *et al.*, 2013). In conclusion, not all

tuberculous lesions were AFB positive possibly because other microorganisms may have been involved.

5.5 Isolation of Mycobacteria

Homogenized tuberculous lesions were cultured on three Lowenstein-Jensen (L-J) culture media enriched with glycerol, pyruvate and para-nitro benzoic acid. Culture medium containing glycerol favours the growth of *Mycobacterium tuberculosis*, while L-J medium containing pyruvate encourages the growth of *Mycobacterium bovis* (Central TB Division and Directorate General of Health Services, 2009). Para-nitro benzoic acid (PNB) inhibits growth of the MTBC but allows other mycobacteria to grow (Giampaglia *et al*., 2007; Nepali *et al*., 2010).

Twenty three percent [23% (50/218)] positive culture was realised in this study. These rates were higher than those observed by Nuru *et al*.(2017) who reported 17.7%, but lower than Sahraoui *et al*.(2009) and Awah Ndukum *et al*.(2010), where 51% culture positive was reported. Among all the isolates, 30 grew on pyruvate, 19 on para-nitro benzoic acid and 10 on glycerol media. Seven isolates grew on both pyruvate and para-nitro benzoic acid and two grew on both glycerol and pyruvate, while one grew in all the three media. Pyruvate reported the highest growth rate of 30 compared with the other two culture media. Only 10 ZN positive lesions had growth on pyruvate. Twenty of the lesions that grew had reported negative result for ZN staining. However some of those lesions that yielded acid fast bacilli did not produce growth on cultivation. There was poor agreement between direct ZN staining and mycobacteria recovery (Kappa=0), indicating that not all ZN positives grew and some of those that turned negative grew. This explains why some ZN positive lesions did not yield growth. In completely calcified lesions, microscopy and staining may yield acid fast bacilli, but organisms being dead and disintegrated, can result to no growth upon cultivation on culture media (Markey *et al*., 2013).

In this investigation 77% (168/218) of the lesions did not yield isolates. This could be due to the involvement of other microorganisms as etiological factors in causing these lesions. Also in completely calcified lesions, mycobacteria are dead and not viable, resulting to no growth upon cultivation on culture media (Markey *et al*., 2013).

5.6 Molecular identification of Mycobacteria

Thirty five out of 1000 sampled cattle carcasses (35/1000) were positive for mycobacteria on PCR and with a prevalence proportion of 3.5% [95% CI: 2.53-4.83]. GenoType[®] *Mycobacterium* MTBC, GenoType[®] *Mycobacterium* CM and GenoType[®] *Mycobacterium* AS were used to characterize mycobacteria isolates. PCR analysis identified Mycobacteria in 35/50 (70%) of the isolates. PCR GenoType *Mycobacterium* (CM) kit identified *Mycobacterium* fortuitum 12/50 (24%) and *Mycobacterium interjectum* 2% (1/50). AS kit identified *Mycobacterium shimoidei* 2/50 (4%), *Mycobacterium celatum*, *Mycobacterium szulgai*, *Mycobacterium asciaticum*, *Mycobacterium kansaasii* 2% (1/50) each, and *Mycobacterium species* 26% (13/50) were not speciated. GenoType[®] MTBC identified *Mycobacterium bovis* subsp.bovis 6% (3/50). The 13 MOTTs that could not be speciated may not be the commonly encountered mycobacteria since the kits are optimized for detecting only 37 Mycobacteria that are commonly encountered.

Zoonotic mycobacteriosis was 0.3% (3/1000) [95% CI: 0.1-0.88] which agrees with Habarugira *et al*., (2014), that reported 0.5%; but was lower than Gathogo *et al*.,(2012); that reported 2.05%. However this was higher than Shitaye *et al*., (2006) that reported 0.03%. The identification of *Mycobacterium bovis* subsp.*bovis* (0.3%) at 95% [CI: 0.1-0.88] as causative agents of mycobacteriosis in cattle in this study was in agreement with other studies done in Africa.

The Mycobacteria Other than Tuberculosis (MOTTs) were identified in this study were: *Mycobacterium fortuitum*, *Mycobacterium shimoidei*, while *Mycobacterium interjectum*, *Mycobacterium asciaticum, Mycobacterium celatum, Mycobacterium szulgai* and *Mycobacterium kansaasii*. In a previous study done in Kenya by Gathogo, it documented the presence of MOTT at 24.4% of those lesions that yielded culture though they were not characterised (Gathogo *et al*., 2012). This study reported a higher figure of 64% (MOTTs) of all the mycobacteria reported, which was higher than reported by Gathogo *et al*., (2012). Two other studies done in Chad and Uganda isolated MOTTs from more than 40% of the animals exhibiting granulomatous lesions (Diguimbaye-Djaibé *et al*., 2006; Oloya *et al*., 2007).

The overall prevalence of mycobacteriosis was 3.5% (35/1000) [95% CI: 2.53-4.83], which was in agreement with Ameni *et al*.(2010) and Demelash *et al*.(2009) that reported prevalence of between 3.5% to 10.2%, Shitaye that reported 3.46% and Biffa who reported 4.53% (Shitaye *et al*., 2006; Biffa *et al*., (2010) , all from Ethiopia. However these findings disagreed with Gebremedhin and Akinaw from Dilla municipal abattoir, Ethiopia, who reported 2.7% and 2.9% respectively (Akinaw, 2016; Gebrezgabiher *et al*., 2014). In this investigation, *Mycobacterium bovis* subsp.*bovis* and seven species of MOTTs were found to be causes of mycobacteriosis in cattle in the study area.

5.7 Public health importance of Mycobacteria identified

This study identified *Mycobacterium bovis* subsp.*bovis* 0.3% (3/1000) [95% CI: 0.1-0.88] as one of the causative agents of bovine tuberculosis. Bovine tuberculosis is of public health importance and concern worldwide, especially in developing countries where preventive measures are inadequate or lacking (Humblet *et al*., 2009). Zoonotic TB in cattle is mainly caused by *Mycobacterium bovis* subsp.*bovis* and can be acquired by humans by eating of raw or undercooked meat has also been implicated (Wedlock *et al*., 2002). Ameni *et al*.(2003) showed that 99% of cases were caused by raw meat or undercooked meat consumption versus

1% of only undercooked meat consumption in Central Ethiopia. Also inhalation of cough droplets sprayed from infected cattle, or from handling or consumption of milk contaminated with the microorganisms has been implicated (Wedlock *et al*., 2002). High prevalence of HIV/AIDS cases causing immunosuppression makes the infected vulnerable to *Mycobacterium bovis* subsp.*bovis* and other opportunistic infections (Grange, 2001).

In developing countries contribution of *Mycobacterium bovis* subsp.*bovis* to TB incidences in human has not been determined accurately due to such factors as under-reporting, inefficiency in diagnosis due to diagnostic limitations of many laboratories (Jiang *et al*., 2015). Pathogenesis of mycobacteriosis in humans differ, depending upon the species and hosts involved and upon ways of infection and may present as pulmonary, skin or soft tissue lesions (Griffith, 2010; Jarzembowski and Young, 2008; Wagner and Young, 2004). Signs in human, include fever, night sweats, weight loss, cough if lungs are involved, abdominal pain and diarrhea if digestive system is involved. In some cases, generalized mycobacteriosis may occur. According to Office International des Epizooties (OIE, 2009), the standard control measure applied to mycobacteriosis is test and slaughter. Mycobacteriosis eradication programs consisting of post-mortem meat inspection, intensive surveillance including on-farm visits, systematic individual testing of cattle and removal of infected and in-contact animals as well as movement controls have been very successful in reducing or eliminating the disease (Etter *et al*., 2006).

MOTTs are commonly found in environmental sources such as water and soil and have a high survival rate (Hruska *et al*., 2012). Most of *Mycobacteria* are opportunistic microorganisms for human and live under various environmental conditions (Converse, 2007). The rapidly growing mycobacteria, *Mycobacterium fortuitum, M. chelonae,* and *M. abscessus* are also opportunistic microorganisms, normally found in soil, dust, and water pipes. According to Wagner and Young, (2004), MOTTs are important environmental

pathogens that can cause a broad spectrum of diseases in humans and animals. MOTTs are potentially pathogenic mycobacteria and have been implicated in cases of mycobacteriosis in humans and animals (Polaček and Aleksić-Kovačević, 2016), can be transmitted from animals to humans, especially in immunocompromised persons. Exposure has different public health implications; in humans they are capable of causing pulmonary disease, disseminated disease or localized lesions in both immunocompetent and immunocompromised individuals (Kankya *et al.*, 2011).

Seven species of the MOTTs were identified in this study (*Mycobacterium fortuitum, Mycobacterium interjectum, Mycobacterium shimoidei, Mycobacterium asciaticum, Mycobacterium celatum, Mycobacterium szulgai* and *Mycobacterium kansaasii). Mycobacterium fortuitum, Mycobacterium interjectum, Mycobacterium shimoidei, Mycobacterium asciaticum, Mycobacterium celatum, Mycobacterium szulgai* and *Mycobacterium kansaasii).* The isolation of MOTTs from carcasses with TB-like lesion signifies the importance of these bacteria in communities but also gives an indication of the mycobacterial status of the environment and water sources (Hruska *et al.*, 2012).

M. fortuitum is a known isolate of the soil and river water (Bland *et al.*, 2005; Hruska *et al.*, 2012). *M.fortuitum* was identified in 12/50 (24%) of the specimens and is one of the mycobacterium avium complex (MAC) species. Infection from this complex has been implicated to cause severe conditions in human and fish mycobacteriosis. MAC infections are also zoonotic transmittable from environmental reservoirs to domestic animal and human (Hibiya *et al.*, 2007). Specifically, in human *M.fortuitum* has been associated with cutaneous and deeper infections after trauma, pulmonary disease and corneal infections (Agheli *et al.*, 2006). Other syndromes in human include osteomyelitis, joint infections, lymphadenitis and endocarditis. Most of these syndromes have been reported in immunosuppressed and especially due to HIV/AIDS (Agheli *et al.*, 2006).

In human *M. celatum* has been recognised as a causative agent of pulmonary and disseminated infections in immunosuppressed humans (Pate *et al*., 2011), especially with pre-existing lung infection. *Mycobacterium shimoidei* also an opportunistic pathogen, has been previously isolated only among subjects with pre-existing lung diseases such as emphysema, previous tuberculosis, silicosis, lung carcinoma, (Galizzi *et al*., 2013) and severely immunocompromised (Galizzi et al., 2013). *Mycobacterium shimoidei*, was first isolated from a respiratory infection in a Japanese patient in 1975.

M.interjectum has been well documented as a source of infection in children and immunocompromised adults; however, there are no descriptions of disease in healthy individuals. It was subsequently reported in 9 pediatric cases of necrotizing lymphadenitis (Mirant-Borde *et al*., 2013). *M.szulgai*, is a relatively uncommon MOTT, accounting for less than 1% of all MOTTs isolations. *M szulgai* was first isolated in humans in 1972 (C and Kibaroglu, 2014). Diseases caused are pulmonary, cervical lymphadenopathy, carpal tunnel syndrome, cutaneous infections, osteomyelitis and less common disseminated diseases (C and Kibaroglu, 2014). Some NTM species, like *Mycobacterium kansasii, Mycobacterium marinum*, and *M. fortuitum* have been implicated to induce anti-mycobacterial immune responses that interfere with the diagnosis of bovine tuberculosis either by tuberculin intra-dermal test or gamma interferon tests (Vordermeier *et al*., 2007).

This study demonstrated the presence of zoonotic tuberculosis (*Mycobacterium bovis* subsp.*bovis*) and pontentially pathogenic species of MOTTs, that though they rarely cause diseases in humans, can infect immunocompetent and immunocompromised individuals (Snydman *et al*., 2010). Previous studies on zoonotic tuberculosis have concentrated mainly on *Mycobacterium bovis* subsp.*bovis* (bovine zoonotic tuberculosis) neglecting the presence and significance of MOTTs. This study has established the zoonotic potential of MOTTs

concluding that research on zoonotic tuberculosis should not be restricted to *Mycobacterium bovis* subsp.*bovis*.

5.8 Economic Importance of Mycobacteriosis in Kenya

This study assessed losses due to condemnations of organs/tissues at the slaughter house level that totalled to 422 kilograms. Organs condemned were mainly lungs, followed by livers and muscle tissues. At the time of this study a kilogram of beef was selling at Kenya shillings 400. The estimated loss stood at Kenya shillings 168,800 KSHS for the four month study period in one major abattoir in Laikipia County. This was estimated at Kenya shillings 506,400 (506 USD) per annum.

The economic impact of mycobacteria infection on livestock production is extremely difficult to determine accurately. The most obvious losses from mycobacteriosis in cattle are direct productivity losses (reduced benefit), which can be categorized into slaughter losses. Slaughter losses comprise the cost of organ and tissue condemnation and with the loss from condemnation being essentially the purchased value of a slaughtered carcass (Bemrew A. *et al*., 2015). Continuous condemnation of tissues and organs found with TB-like lesion reduces the amount and quality of meat reaching the market.

Desktop study considered trade inquiries in the last 10 years at the national level, where 196 inquiries for trade in animals for meat and animal products were received. Eighty percent (80.6%) were secured while 19.4% failed and the main reason for failure was inability to meet minimum required threshold for tuberculosis control measures. Of the two enquiries that failed, an estimation value of Kenya shillings 405 million (4.05 million USD) was lost due to failure to demonstrate zoonotic tuberculosis surveillance and control programmes.

At the local level, the disease reduces livestock productivity in general and may be economically devastating for the cattle industry especially the dairy sector (Zinsstag *et al*.,

2006). These losses include those related to animal production, markets and trade, as well as the costs of implementation of surveillance and control programmes. Most important is the impact of the risk of infection to humans, particularly for women and children who are most exposed to the disease in countries with poor economic conditions and weak veterinary and public health services.

Another important loss is the infection with environmental non-pathogenic mycobacteria (MOTTs) causing test for tuberculosis to be positive (Watchman *et al*., 2011) though they may not qualify as zoonotic tuberculosis however the test results are used to prevent Kenyan cattle from accessing the international market. Internationally trade is hindered when positive reactors are found among animals to be exported. Although estimates of the costs associated with mycobacteriosis and its control refer only to specific countries, all data suggest that worldwide economic losses due to the disease are significant. In general mycobacteriosis affects the national and international economy in different ways (Bemrew *et al*., 2015), which has major implications for other economic sectors, which are linked to livestock production.

In conclusion, this study has established that slaughter house condemnation of organs/tissues and restriction of animal trade due to bovine mycobacteriosis impact negatively on the national economy and international livestock trade.

5.9 Conclusions

1) The current study reported a prevalence of 21.8% on macroscopic lesions identification, however the study reported a lower prevalence than the conventional of 47% reported in France; this may have been caused by the high proportion of small undetected lesions coupled with the few numbers of tissues inspected in routine carcass and detailed inspections.

2) According to the results in this study, majority of the lesions were found in the lungs and associated lymph nodes (57.8%), which may indicate higher aerosol infection route than oral

(ingestion) route. This could be due to nomadic transhumance production system, where livestock are moved together as several herds to follow grazing and water during drought seasons, thus increasing contact between animals and raising chances of infection.

3) In this investigation majority of the lesions were calcified (46.8%), indicating a high level of immunity of animal and the long duration of the infection.

4) The fact that a large number of tuberculous lesions turned ZN negative, means other microorganisms may have caused these lesions.

5) In this investigation 77% (168/218) of the lesions did not yield isolates. This could be due to the involvement of other microorganisms as etiological factors in causing these lesions. Also in completely calcified lesions, mycobacteria are dead and not viable, resulting to no growth upon cultivation on culture media.

6) This study demonstrated *Mycobacterium bovis* and seven species of MOTTs as the causative agents of mycobacteriosis in cattle in the study area.

7) This study has established the zoonotic potential of MOTTs concluding that research on zoonotic tuberculosis should not be restricted to *Mycobacterium bovis* subsp.*bovis*.

8) This investigation has established that slaughter house condemnation of organs/tissues and restriction of animal trade due to bovine mycobacteriosis impact negatively on the national economy and international livestock trade.

6.0 Recommendations

1) Detailed inspection of bovine meat carcasses should be emphasized to reduce transmission of zoonotic tuberculosis and other mycobacteriosis to humans through meat.

2) Regular sample taking especially of suspected tuberculous lesions by the meat inspectors for detailed laboratory examinations is recommended for detection of bovine tuberculosis.

3) Mycobacteriosis research and control should be prioritized now that there is a threat of the disease among our cattle population.

4) Further research on zoonotic tuberculosis should take into account the pathogenic potential of Mycobacteria Other than Tuberculosis (MOTTs).

5) Considering that there is antigenic cross-reaction between mycobacteria species, the effect of MOTTs on the accuracy of immunodiagnostic tests such as the tuberculin tests need to be examined.

6) Surveillance and control programmes for mycobacteriosis in cattle are important in order to reduce economic losses and protect human health.

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